Southeastern Fishes Council Proceedings

Number 53 (December 2011)

12-1-2011

Number 53 (December 2011)

Southern Fishes Council

Number 53 (December 2011)

Abstract
(December 2011) - Population Status and Environmental Associations of the Rare Striated Darter, Etheostoma striatulum. By Andrew C. Abernathy and Hayden T. Mattingly

Conservation Status of the Longhead Darter, Percina macrocephala, in Kinniconick Creek, Kentucky. By David A. Eisenhour, Audrey M. Richter and Joshua M. Shiering

First Observation of a Natural Hybrid Between Endangered Roanoke Logperch (Percina rex) and Chainback Darter (Percina navisense). By James H. Roberts

Minutes, Business Meeting, 36th Annual Meeting, Southeastern Fishes Council

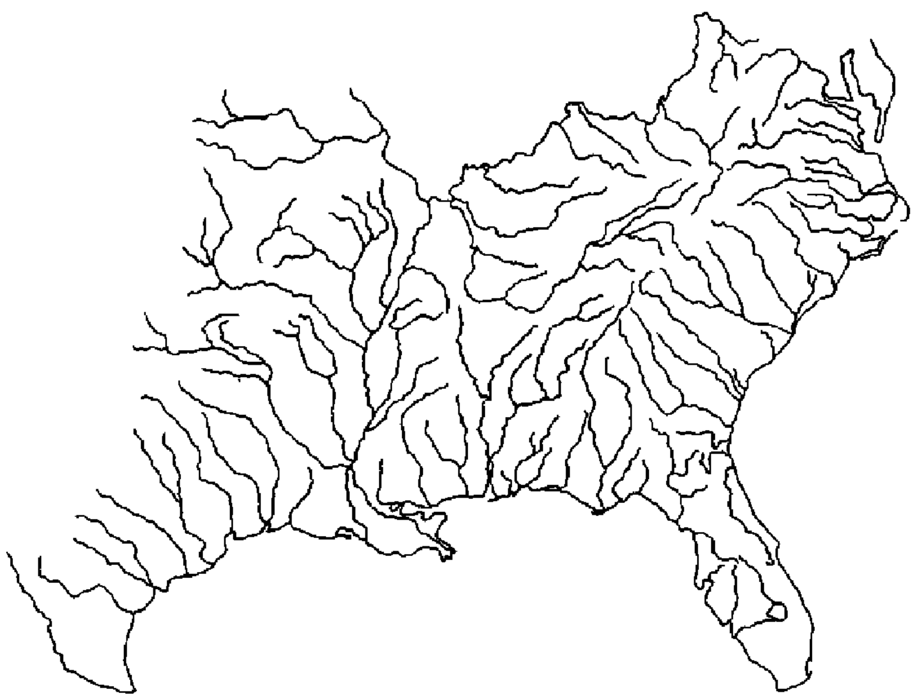
2010 Treasurer's Report for the Southeastern Fishes Council

Keywords
fishes, population, environmental, conservation status, longhead darter, percina macrocephala, kinniconick creek, kentucky, hybrid, roanoke logperch, percina rex, chainback darter, percina navisense

Number 53 December 2011

Southeastern Fishes Council Proceedings

Dedicated to the Conservation of Southeastern Fishes



CONTENTS

Annual Dues

Please check your mailing label. Opposite your name on the mailing label is the last year for which we have a record of dues paid (or "9999" for lifetime members). If you have not yet paid 2011 dues (or other dues in arrears), please send a check payable to Southeastern Fishes Council to Steve Rider, Alabama Wildlife and Freshwater Fisheries Division, 64 N. Union Street, Suite 551, Montgomery, AL 36130, (334) 844-8978. Dues are $30.00 per year for regular members and $15.00 per year for students. If you are uncertain regarding your status, email Steve Rider at Steve.Rider@dcnr.alabama.gov.

37th Annual Meeting, Southeastern Fishes Council

The SFC met in Chattanooga, TN on Thursday and Friday, November 9-10, 2011. The meeting program and abstracts are posted on our website <www.sefishescouncil.org>.

Student Award Winners

Oral Presentations

1st Place - Loren Stearman, University of Central Arkansas. Life history of the redfin darter, *Etheostoma whipplei,* in central Arkansas (with Ginny Adams)

2nd Place - Brook Fluker, University of Alabama. Spring-adapted species as a model for understanding the genetic consequences of aquatic habitat fragmentation (with Bernard R. Kuhajda, and Phillip M. Harris)

3rd Place - Mark Hoger, Austin Peay State University. Inter-seasonal movements of Etheostomatinae darters in Yellow Creek and Whiteoak Creek, Tennessee (with Rebecca Blanton)

Poster Presentations

1st Place - John Johansen, Tennessee Tech University. Development of species-habitat models to inform conservation planning for freshwater species covered by the Cumberland Habitat Conservation Plan (with Hayden Mattingly)

2nd Place - Laura Stewart, University of Southern Mississippi. Body shape and burst-swimming performance in the *F. notatus* complex: A tale of two tails (with Nathan R. Franssen and Jake Schaefer)

3rd Place (tie) - Zach Martin, University of Florida. A preliminary investigation of the morphological diversity of genital papillae in *Etheostoma* and its association with spawning behavior

3rd Place (tie) - Matthew Wagner, Austin Peay State University. Utility of amplified fragment length polymorphisms in a phylogeographic study of the redband darter, *Etheostoma luteovinctum* (with Rebecca Blanton)

3rd Place (tie) - Christopher Yates, Kennesaw State University. The effects of urbanization on food sources and gut morphology in largescale stoneroller (*Campostoma oligolepis*) (with Troy Mutchler and William Ensign)

38th Annual Meeting, Southeastern Fishes Council

The SFC will meet on Thursday and Friday, November 8-9, 2012 in New Orleans, LA. On Saturday, November 10 there will be a memorial dedicated to the life of Dr. Royal D. Suttkus. Please check our website <www.sefishescouncil.org> periodically for updates.

Population Status and Environmental Associations of the Rare Striated Darter, *Etheostoma striatulum*

ANDREW C. ABERNATHY[1] AND HAYDEN T. MATTINGLY[2]*

[1]Tennessee Stream Mitigation Program, 5000 Lindbar Dr., Suite 265, Nashville, TN 37211 USA

[2]Department of Biology, Box 5063, Tennessee Technological University, Cookeville, TN 38505 USA

**Corresponding author*: hmattingly@tntech.edu

ABSTRACT

The Striated Darter, *Etheostoma (Catonotus) striatulum*, is a rare percid whose known range is restricted to 15 streams in the mid-to-upper Duck River system, Tennessee. The last rangewide assessment of its conservation status occurred in 1992, a survey which yielded only 26 specimens. In June-July 2006 we reevaluated the darter's population status and characterized its habitat by surveying 30 reaches in 22 streams. Striated Darters were detected in 11 of 30 reaches with a total of 102 individuals observed; 78 were young-of-year juveniles and 24 were adults. In late July total lengths ranged 19-31 mm (mean = 24 mm) for juveniles and 39-49 mm (mean = 43 mm) for adults, with only two age classes indicated. Several new occupied reaches were identified in streams where the darter had been collected previously; however, four historically occupied reaches, including the type locality, failed to produce specimens. At one of the better sites, a mark-recapture experiment revealed a population estimate of 136 Striated Darters per 100 m at a density of 0.14 individuals per m^2. The 11 occupied reaches had the following mean characteristics: elevation 215 m above sea level, stream order 4.3, link magnitude 65, riparian zone width 11 m, wetted channel width 11 m, mid-channel depth 25 cm, discharge 0.01 m^3/s, water temperature 26 C, pH 8.0, and conductivity 247 µS. On average, approximately one-fourth of the bottom surface of runs and pools was covered with broken slabrock substrate in the 11 occupied reaches. The number of individuals of other *Catonotus* species (*E. flabellare*, *E. crossopterum* and *E. nigripinne*) observed in occupied reaches was highly variable but averaged 53 individuals per reach. Environmental characteristics of occupied reaches were not significantly different from those of unoccupied reaches, at least for variables measured in this study. Our results highlight a species with limited distribution and abundance, a short lifespan, and whose status parallels that of other imperiled *Catonotus* species.

INTRODUCTION

The Striated Darter, *Etheostoma striatulum* Page & Braasch, is a small *Catonotus* darter of the *E. virgatum* group found within the Nashville Basin physiographic region in Tennessee (Fig. 1). The darter's known range spans parts of the mid-to-upper Duck River drainage in Bedford, Coffee, Lewis, Marshall, and Maury counties, with occurrence records in only 15 streams (Page and Braasch, 1977; Page, 1980; Cook et al., 1996). Other *Catonotus* species found in the drainage include *E. flabellare*, *E. smithi*, *E. crossopterum* and *E. nigripinne* (Etnier and Starnes, 1994).

Etheostoma striatulum is a small-bodied darter that reaches a recorded maximum standard length of 47 mm (Cook et al., 1996). It is considered to be an annual species, with the oldest observed specimen in Page's (1980) life history study aged at 17 months (a female). Page (1980) observed that all April-collected males were one year old and in spawning condition. He also documented rapid growth, with males and females reaching half of their maximum body size in the first three months of life.

Little is known about the Striated Darter's population status and environmental associations due to the paucity of research conducted on the species since its formal description 30+ years ago. Page and Braasch (1977) examined 89 specimens when describing the species, and Page's (1980) life history study utilized 191 individuals harvested over a two-year period, December 1976 to January 1979. Cook et al. (1996) conducted a status survey in 1992 encompassing all known historical collection localities. Only 26 specimens were collected from a total of 10 sites, illustrating Page's (1980) belief that the darter was a "generally uncommon" species. Further, Etnier and Starnes (1994) noted that their collection efforts at several historic sites had produced no additional specimens.

Anthropogenic disturbances in the Duck River system are likely affecting the distribution and abundance of what may be a naturally rare species. Tennessee Department of Environment and Conservation (as of 2001) identified 18 "potentially unsafe" and another 13 "impaired" stream segments in the Duck River drainage. Cook et al. (1996) listed the most prevalent threats as stream alterations, runoff from livestock pastureland, and siltation from agricultural practices. In addition, annual species like the Striated Darter are particularly vulnerable to adverse habitat modifications by sudden events such as extreme flow conditions or chemical spills. Such disturbances could conceivably inhibit or prevent spawning in a given year, thereby undermining the viability of local populations. The darter is considered threatened in Tennessee but receives no federal protection.

The few individuals observed within Striated Darter populations, combined with a small geographic range, make this species a prime candidate for implementation of conservation practices. Such implementation will benefit from current information on population status and environmental conditions in Striated Darter streams. Therefore, the objectives of this study were to (1) determine population status of the darter at historic and potential new sites across its range, (2) conduct a population estimate at one occupied site, and (3) relate the darter's presence or absence to environmental variables measured at all sites.

METHODS

Population Status

Thirty, 100-m reaches were sampled during June and July 2006 (Table 1, Fig. 2) with an emphasis on revisiting sites in the Cook et al. (1996) study and new locations that could support Striated Darter populations. Detailed site descriptions and coordinates are provided by Abernathy (2007). Each reach was divided into five, 20-m sections. Most sampling was conducted using seining methods with the exception of four deeper sites where seining was relatively ineffective; these sites (8, 27, 28, and 29) were sampled by snorkeling. The Duck River proper was sampled at Henry Horton State Park (7) although not under the standard seining or snorkeling protocol used at the other 29 sites because of the different stream conditions found in the Duck River mainstem (see Abernathy, 2007).

At the beginning of the survey we compared the relative effectiveness of collecting *Catonotus* darters with seining versus backpack electrofishing at Site 11 in Butler Creek. Seining produced more individuals representing a wider range of body sizes than did electrofishing. We also wanted to avoid potential injuries to darters that might be induced by electrofishing. For these reasons our primary sampling technique was seining.

The standard seining protocol consisted of two persons using short seine hauls while vigorously kicking and disturbing the substrate. The seine dimensions were 1.2 m x 3.0 m and the mesh size was 3.2 mm. All *Catonotus* darters, including the Striated Darter, were removed from the stream, counted, identified to the lowest possible taxonomic level, and placed in separate aerated buckets until sampling in that 20-m section was completed. Our protocol did not include the Cook et al. (1996) "set-kick" seining technique of encircling single slab rocks.

We devised a system to determine how many seine hauls would be conducted in a given reach section. First, the mean width of each 20-m section was obtained by averaging two random wetted channel width measurements taken within the section. Sections that averaged ≤5 m in width were given five seine hauls. The number of seine hauls increased as mean section width increased: a section 6-10 m wide was sampled with 10 seine hauls; 11-15 m wide, 15 seine hauls; 16-20 m wide, 20 seine hauls; and a section 21-25 m wide, 25 seine hauls.

The snorkeling protocol was to thoroughly examine the substrate within the given stream reach. This process consisted of two persons slowly and deliberately moving upstream through the reach in a zigzag fashion. Snorkeling effort ranged from 90—110 min per reach.

Population Estimate

A population estimate was conducted at one site, Flat Creek at Hwy. 64 (Site 2, Table 1, Fig. 2), using the Petersen mark-recapture method on 25-26 July 2006. A pilot study of mark-recapture procedures was conducted at two other sites (13, 15). Population estimate procedures followed Martin et al. (1999) except that block nets were placed at the upstream and downstream boundaries of each site. Striated Darters collected on the first day were anesthetized with 20 mg/L clove oil, marked by cutting a small amount of tissue from the lower portion of their caudal fin, revived in freshwater until normal fin and opercular movement was observed, and then placed back in the stream randomly throughout the 100-m reach. The reach was then resampled 24 h later to count numbers of marked and unmarked Striated Darters. Furthermore, Striated Darters at sites 2, 13, and 15 were measured to the nearest mm total length (TL) on 24-26 July 2006 to enable construction of length-frequency histograms and delineate population age-class structure.

Population estimates were established for both age-0 (<32 mm TL) and age-1+ (>32 mm TL) classes using the formula $N = (MC)/R$, where N = the population estimate, M = number of individuals marked on the first day, C = number of individuals collected on the second day that were not marked on the first day, and R = number of individuals marked on the first day subsequently recaptured on the second day. Striated Darter density was calculated by dividing the population estimate by the surface area of the site; surface area was determined by multiplying the mean width of each 20-m section by its length and summing these five areas. An estimate of seining gear efficiency was obtained by dividing initial catch on the first day by the population estimate (N) calculated for the site.

Environmental Variables

Striated Darter presence or absence was related to 12 environmental variables measured at the 30 sites. Elevation, stream order, and link magnitude (Osborne and Wiley, 1992), were determined using contour lines on U.S. Geological Survey topographic maps (1:24,000 scale) and Maptech software (Terrain Navigator, version 6.02); only perennial streams were included when calculating stream order and link magnitude. Riparian zone width (RZW) was evaluated by visually estimating the extent of woody vegetation on both sides of the stream to generate an average one-side-only RZW; tape measurements were taken occasionally to check visual estimates. Mean mid-channel depth was determined by taking three mid-channel readings per 20-m section (no redundant readings) and averaging the 15 values; mean wetted channel width was obtained by averaging two channel widths per 20-m section as described above for "Population Status". Discharge was measured once per reach using a Marsh-McBirney Flo-Mate 2000 and top-setting wading rod (McMahon et al., 1996). Water temperature and conductivity were measured with a YSI Model 85 meter, and pH was measured with an Oakton Instruments pH Testr 3+ meter.

The amount of microhabitat available for Striated Darters was ranked categorically for each 20-m section in a reach; these five ranks were then averaged to generate a single index of available microhabitat for each reach. Microhabitat categories were based on visual estimates of the percent of loose stone microhabitat present in runs and pools within the section (riffle areas were excluded). Category ranks were: 0 = <5% available stone habitat; 1 = 6-25% available habitat; 2 = 26-50% available habitat; 3 = 51-75% available habitat; and 4 = 76-100% available habitat. The accuracy of the visual-estimation method was checked in early August 2006 at two representative sites outside the suite of sites canvassed in this study.

Other *Catonotus* species collected in the study reaches were handled in the same manner as the Striated Darter. Individuals were classified as "unknown spottails" if they were *E. crossopterum* or *E. nigripinne* because distinguishing non-nuptial individuals of these two species can be very difficult (see Page et al., 1992). Abundance of other *Catonotus* was calculated by summing the number of spottails (*E. crossopterum* and/or *E. nigripinne*) plus *E. flabellare* to generate a total per 100-m reach.

Statistical Analysis

Frequency histograms were constructed separately for reaches where Striated Darters were present versus reaches where they were absent by breaking continuous environmental variables into discrete intervals, or by following existing intervals for categorical variables. Each environmental variable was analyzed using Fisher's Exact Test in SAS version 8.2 (SAS Institute, 1995) to determine whether frequency distributions of presence versus absence reaches differed from one another. For all statistical analyses, = 0.1.

RESULTS

Population Status

The Striated Darter was present at 10 of 26 seining reaches and one of four snorkeling reaches (Table 1, Fig. 2). The darter's continued presence was confirmed at six of the 10 occupied sites identified by Cook et al. (1996) in their 1992 survey; however, it was not found in four previously occupied sites including the type locality (1, 6, 7, 8). The darter was found in five reaches (13, 14, 15, 22, 27) in 2006 that were not sampled in 1992. Sites 13, 14 and 15 were new location records in streams known to be inhabited elsewhere by the species in 1992 (Fig. 2). Striated Darters were collected in Alexander Creek in 1937 and Noah Fork in 1962; our sampling at sites 22 and 27 reaffirmed their presence in these streams, albeit at different locations than the historic records.

One hundred and two Striated Darters were collected in the 11 occupied reaches (Table 1); 78 of these individuals were young-of-the-year juveniles <32 mm TL and 24 were considered age-1+ adults, yielding a juvenile-to-adult ratio of 3.3 to 1 (this ratio was heavily influenced by 42 juveniles observed at Site 13). Length-frequency analysis in late July confirmed a distinct separation between the two age classes (Fig. 3). Age-0 juveniles (N = 88) at three sites averaged 23.5 ± 4.9 mm TL and ranged 19—31 mm TL, while age-1+ adults (N = 35) averaged 42.8 ± 1.7 mm TL and ranged 39—49 mm TL. The juvenile-to-adult ratio at these three sites was 2.5 to 1, slightly lower than that calculated for the wider survey.

Population Estimate

In Flat Creek (Site 2), 14 individuals were collected and 13 were marked on the first day, and 21 individuals were collected on the second day with two of these being recaptures (one juvenile and one adult). The population estimate for both age classes combined was 136 Striated Darters per 100 m at a density of 0.14 individuals per m^2. Population estimates for age 0 and age 1+ individuals per 100 m were 32 and 117 individuals, respectively. Seining gear efficiency at Site 2 was 10%, indicating that only one in 10 Striated Darters was vulnerable to the gear at this particular site.

Environmental Variables

The 11 reaches occupied by Striated Darters had the following mean characteristics: elevation 215 m above sea level, stream order 4.3, link magnitude 65, riparian zone width 11 m, wetted channel width 11 m, mid-channel depth 25 cm, discharge 0.01 m^3/s, water temperature 26 C, pH 8.0, and conductivity 247 µS (Table 2). On average, approximately one-fourth of the bottom surface of runs and pools was covered with broken slabrock substrate in occupied reaches (mean index of available microhabitat was 1.5). The number of individuals of other *Catonotus* species observed in occupied reaches was highly variable but averaged 53 individuals per reach.

Environmental characteristics of occupied reaches were not significantly different from those of unoccupied reaches (Table 2; Fisher's Exact Test; all $P \geq 0.20$). Frequency distributions for most variables associated with Striated Darter presence mimicked those associated with darter absence.

DISCUSSION

Population Status

Our study represents the first rangewide assessment of Striated Darter populations using a standardized protocol at sites of fixed length (100 m). As such, our data provide a numerical baseline at 25 seined sites to which future population monitoring data can be compared. The previous survey in 1992 by Cook et al. (1996) differed from our survey in a number of ways. First, Cook et al. surveyed during May, July, and October whereas we sampled in June and July. Second, Cook et al. used an unspecified mixture of set-kicks and standard seine hauls, whereas we used only the latter. A set-kick involved placing the seine around a single slab rock, lifting the slab with two persons kicking the substrate, thereby "chasing" the fish into the net. The set-kick technique specifically targeted a habitat feature presumed likely to yield Striated Darters: slab rocks over bedrock. Third, Cook et al. did not standardize sampling effort at each site. For example, the number of set-kicks and seine hauls (collectively termed "attempts") in their survey varied from 1 to 25 attempts at the ten sites occupied by the darter. Finally, Cook et al. did not estimate the efficiency of their sampling methods. We found our seining efficiency to be 10% (determined from data at a single site) which suggests a fairly high probability that Striated Darters went undetected at one or more sites where they were actually present in low numbers.

Despite the differences between the 1992 and 2006 surveys, some broad patterns in Striated Darter population status are apparent. In both surveys Striated Darter presence was confirmed at only 10-11 stream reaches and only 24-26 adults were observed (assuming the 26 specimens reported by Cook et al. were adults). The species is clearly a rare fish with a limited range and we concur with Etnier and Starnes (1994) and Cook et al. (1996) that Striated Darter populations are quite vulnerable to depletion or extirpation.

Six of the 10 sites where Cook et al. (1996) found Striated Darters produced individuals during our survey (Fig. 2). Only one of these six sites, however, yielded >10 individuals (Site 9). This reach of Flat Creek provided ample habitat conditions for Striated Darters. Most of the 16 individuals were collected around pool margins, often where broken slabrock was found abutting *Justicia* sp. beds. Upstream of Site 9 in Flat Creek, Site 13 produced the three highest Striated Darter counts seen in this study. Three separate sampling events produced 44, 57, and 152 Striated Darters (the latter two counts were obtained during the population estimate pilot study). This site contained very little broken slabrock and only modest amounts of *Justicia* sp. Most individuals were juveniles collected over open bedrock. Much more of what is perceived to be optimal *Catonotus* habitat (i.e., more broken slabrock present) can be found both upstream and downstream of Site 13.

Three new occurrences were identified in streams known to be inhabited by *E. striatulum* (Sites 13, 14, and 15) and new occurrences were noted for two additional streams, Alexander Creek (22) and Noah Fork (27). Striated Darters had been collected in both streams prior to 1992, yet were not collected during the 1992 survey. The easternmost (Site 27) and westernmost (Site 10) sites where *E. striatulum* was encountered are outliers not only in geography but also geology. These two sites show both Nashville Basin and Highland Rim qualities. Both streams contain more cherty gravel than do the remainder of the streams sampled. Most of the streams sampled within the interior Nashville Basin display a prominently bedrock substrate intermingled with patches of gravel and cobble. Gravel and cobble are much more prevalent in both Noah Fork and West Fork of Bigby Creek. Only one individual Striated Darter was collected at each site. It may be that Striated Darters were never common in either drainage due to habitat restrictions, such as less available loose, broken slabrock over bedrock. It would be interesting to see if more intensive sampling in optimal habitat within these two streams produces more *E. striatulum* specimens. As one proceeds downstream through the Noah Fork and Bigby Creek drainages, the nature of these streams tends to shift from Highland Rim origins towards qualities representative of interior Nashville Basin streams. The focus of Striated Darter studies within these two streams may need to be shifted accordingly.

Population Estimate

Our population estimate of 0.14 Striated Darters per m^2 at Site 2 in Flat Creek was intermediate between the Cook et al. (1996) estimate of 0.04 per m^2 at Site 3 in Hurricane Creek and the Page (1980) estimate of 1.34 per m^2 at Site 1 in Wartrace Creek. The density at Site 1 calculated by Page (1980) was only in the slabrock portion of a large pool at the type locality, and thus probably represents a value near the high end of the range of abundances exhibited by the species.

Through our population estimate we were able to generate a rough estimate of our seining efficiency at Site 2. Future research should examine collecting gear efficiency at multiple sites to better place survey results in context. It will remain important to understand detection probabilities, especially when monitoring population status in upcoming years.

The pilot study conducted at two sites refined the protocol used in the mark-recapture population estimate for this species. A common dose of clove oil at 40 mg/L (e.g., Detar and Mattingly, 2005) was initially used to anesthetize Striated Darters in the pilot study. This dose proved to be too strong for age-0 individuals to recover and age-1+ individuals required increased recovery time (25 min) before being released back into the stream. All of the individuals that failed to recover from anesthesia were juveniles (<32 mm TL). This could be linked to stress due to handling, anesthesia, fin clipping, or any combination thereof. Regardless, it appears that Striated Darters should be handled with care during any subsequent sampling events. The initial clove oil (anesthesia) concentration of 40 mg/L was reduced to 20 mg/L for the actual mark-recapture experiment. The 20 mg/L dose was sufficient for anesthetizing fish while still allowing recovery in a reasonable amount of time.

Environmental Variables

Cook et al. (1996) reported mean stream widths of 11 m at sites occupied by Striated Darters in their 1992 survey, with a range of 5-20 m. We calculated exactly the same average wetted channel width, 11 m, from our 2006 occupied sites, with a similar range of 5-19 m. In 2006 Striated Darters occupied 1 third-order site, 6 fourth-order sites, and 4 fifth-order sites which reflects this range of stream widths. However, the frequency distribution of wetted channel widths at occupied sites did not differ from that of unoccupied sites (Table 2). In fact, none of the reach-scale environmental variables showed a statistically significant difference, suggesting that (1) other reach-scale variables and (2) variables at different spatial scales should be examined in future studies. Increasing sample size to increase statistical power at the reach scale could help determine whether the trend noted for water temperature is a real phenomenon. However, the apparent limited distribution of the species will ultimately limit sample size at the reach scale.

Darters belonging to the subgenus *Catonotus* are believed to require broken slabrock for spawning and/or habitat. Cook et al. (1996) reported that slab rocks occupied by Striated Darters typically averaged 25 by 22 by 5 cm. However, we were unable to identify a link between amount of microhabitat available and the presence or absence of Striated Darters. Striated Darters were collected at sites with an abundance (>50%) of available microhabitat, as well as at sites with apparently insufficient microhabitat. Many sites also contained beds of *Justicia* sp. Although these stands of *Justicia* sp. were not included in scoring for microhabitat categories, a number of Striated Darters were collected in and around these stands.

A larger temporal scale (other months, seasons, years) should be used when examining habitat preferences of the Striated Darter at different stages during its lifetime. Older individuals are believed to be obligated to loose stones for breeding purposes and appear to use them, as well as *Justicia* sp. beds, for non-breeding habitat. Young-of-the-year individuals may not begin to compete with larger adults for habitat until they reach the point where they are competitive or until their diet has shifted to larger prey that are not found over open slabrock. Page (1980) showed that a decrease in the amount of crustaceans consumed by Striated Darters occurred as individuals became larger, and these were subsequently replaced by larger insects, primarily chironomid larvae.

Werner and Gilliam (1984) proposed that such ontogenetic shifts are common in animals where resource use and predation risk are related to body size. Therefore, reaches with characteristics such as Site 13, where 42 of 44 individuals were juveniles collected over open bedrock, may be utilized by Striated Darters prior to adulthood. These smaller individuals are presumably less competitive than adults at acquiring habitat space, use different prey resources, and therefore may occupy different habitat until they reach a point where they can secure their own stones for habitat and/or nesting. It is also possible that these younger individuals may have been dislocated from upstream through a high-water event and subsequently settled in this area. The Striated Darter is believed to be an annual species with the oldest observed individual being 17 months (Page, 1980). Should adults perish during summer or autumn, it is conceivable that younger individuals may move in and occupy the niche space vacated by senescing individuals. In short, no definitive statements can be made regarding microhabitat due to the number of individuals observed away from what is believed to be optimal adult *Catonotus* habitat. Enough individuals were collected near emergent stands of *Justicia* sp. and over open bedrock to create some uncertainty regarding the nature (obligatory or facultative) of the relationship between Striated Darters and broken slabrock outside of the spawning season.

Etheostoma crossopterum and *E. nigripinne* often appear to be the most dominant benthic species in streams where they occur (Table 1). In many middle and upper Duck River tributaries, one can hardly pull a seine through a slabrock pool or riffle without collecting several specimens of either *E. crossopterum* or *E. nigripinne*. Barcheek darters in the *E. virgatum* group, like the Striated Darter, show very unique distribution patterns and causation behind these distributions has yet to be fully determined. Barcheeks often have large geographic gaps in their distribution and these gaps are often filled in by other barcheeks (Page and Schemske, 1978). Barcheek species are rarely, if ever, collected sympatrically. Barcheek darters are found primarily in the Cumberland River system, yet certain members of this group can be found in the lower Ohio and lower Tennessee systems. *Etheostoma striatulum*, however, is an outlier when compared to other barcheek distributions. *Etheostoma striatulum* occurs in the upper Duck River drainage and apparently has no contact zone with other barcheeks. It is also interesting to note that the middle portion of the Duck River is largely void of barcheeks. There appears to be a substantial amount of suitable habitat within this portion of the drainage, yet something seems to be restricting barcheeks from range expansion into this area. The extreme lower Duck River and its tributaries are often inhabited by *E. smithi*, whereas the upper reaches are occupied by *E. striatulum*.

This biogeographic phenomenon, along with various other aspects of *Catonotus* species' ecology and life histories, may be driven by competitive exclusion (Page and Schemske, 1978). These authors believe that competition among slab-pool species of *Catonotus* appears responsible for their allopatry. Page and Schemske (1978) also speculated that the presence of members of the *E. squamiceps* complex (e.g., *E. crossopterum* or *E. nigripinne*) often appears to drive body-size reductions in other *Catonotus*. These body-size reductions may be in response to the fact that members of the *E. squamiceps* complex are often larger than other *Catonotus* and are more competitive (and thus more successful) in their ability to secure larger and more optimal nesting sites (stones). These competitively inferior *Catonotus* species may be forced to utilize less-than-optimal habitat.

Despite this suggestive evidence in the literature, no link between the number of other *Catonotus* and the presence or absence of the Striated Darter could be established in our study. As mentioned earlier, large numbers of *E. crossopterum* and/or *E. nigripinne* were collected at sites with and without Striated Darters (Table 1). These members of the *E. squamiceps* complex, and more specifically, *E. crossopterum*, may be better at adapting to degraded stream conditions (Strange, 2000). Both *E. crossopterum* and *E. nigripinne* are much larger than *E. striatulum* and should be more competitive at securing habitat space.

Although not quantified in this study, it appeared that at a number of sites either *E. crossopterum* or *E. nigripinne* appeared to be using broken slabrock and other flat objects for habitat, whereas *E. striatulum*, if observed, was often collected around stands of *Justicia* sp. Also, juvenile *E. crossopterum* or *E. nigripinne* appeared to favor filamentous algae mats for habitat instead of open bedrock that juvenile Striated Darters appeared to prefer. Again, more research into the ecological relationships of *E. striatulum* with other members of the subgenus *Catonotus* are necessary to make any definitive statements.

Conservation Implications

This study reaffirmed that *Etheostoma striatulum* is a rare species with a relatively small geographic range. Cook et al. (1996) found only 26 Striated Darters at 10 of 16 historically known collection localities and concluded that the species' range had been diminished as of 1992. Similarly, we only observed 24 adults and 78 juveniles at 11 sites in 2006. A few other *Catonotus* species have similar levels of low abundance, including *E. chienense*, *E. forbesi*, and *E. lemniscatum* (Blanton and Jenkins 2008). Warren et al. (1994) reported collecting 72 *E. chienense* from only five sites in the Obion Creek and Bayou du Chien drainages of western Kentucky. Eisenhour and Burr (2000) observed 71 *E. lemniscatum* at 12 sites in Big South Fork of the Cumberland River, and Hansen et al. (2006) reported 75 adult *E. forbesi* at six sites in the upper Caney Fork River system. Although survey techniques may not be directly comparable among studies, the Striated Darter appears to be as uncommon as these other rare species in its subgenus, two of which (*E. chienense* and *E. lemniscatum*) are federally protected endangered species.

Although no significant differences in environmental associations were elucidated in our study, anecdotal evidence suggests that habitat degradation continues to be problematic in Striated Darter streams. For example, we did not detect the darter at four sites where it was seen in 1992, including the type locality which is in a degraded condition. As noted above, additional research will be required to better understand the threats faced by this species.

The combination of low abundance, small geographic range, non-detection at selected sites, and anecdotal evidence of degraded stream conditions warrants the attention of biologists and policymakers charged with resource conservation duties in the Tennessee region. Regular monitoring of population trends and efforts to protect and restore stream habitat quality would be prudent conservation measures to encourage the persistence of this unique Duck River species.

ACKNOWLEDGMENTS

Financial and other support was provided by Tennessee Wildlife Resources Agency, U.S. Fish and Wildlife Service, The Nature Conservancy, and the Graduate School, Department of Biology, and Center for the Management, Utilization and Protection of Water Resources at Tennessee Technological University. We especially thank Kurt Snider, Geoff Call, Amy Wales, Corey Giles, Clint Borum, Michael Graf, Paul Benton, Tyler Black, and Jacob Westhoff for assistance during the project. Shawna Herleth-King compiled watershed metrics and assisted with manuscript preparation. The manuscript was improved by comments from S. Bradford Cook, Shawna Herleth-King, Trisha D. Johnson, David D. Smith, and two anonymous reviewers.

LITERATURE CITED

Abernathy, A.C. 2007. Population status and environmental correlates of the rare striated darter, *Etheostoma striatulum*, in the Duck River system, Tennessee. Master's Thesis, Tennessee Technological University, Cookeville. 51 pp.

Blanton, R.E. and R.E. Jenkins. 2008. Three new darter species of the *Etheostoma percnurum* species complex (Percidae, subgenus *Catonotus*) from the Tennessee and Cumberland river drainages. Zootaxa 1963:1-24.

Cook, K.M., C.A. Taylor, and B.M. Burr. 1996. Status review and distribution of the Striated Darter, *Etheostoma striatulum*, and Coppercheek Darter, *Etheostoma aquali*, in the Duck River system, Tennessee. Journal of the Tennessee Academy of Science 71:47-55.

Detar, J.E. and H.T. Mattingly. 2005. Response of Southern Redbelly Dace to clove oil and MS-222: Effects of anesthetic concentration and water temperature. Proceedings of the Annual Conference Southeastern Association of Fish and Wildlife Agencies 58:219-227.

Eisenhour, D.J. and B.M. Burr. 2000. Conservation status and nesting biology of the endangered Duskytail Darter, *Etheostoma percnurum*, in the Big South Fork of the Cumberland River, Kentucky. Journal of the Kentucky Academy of Science 61:67-76.

Etnier, D.A. and W.C. Starnes. 1994. The Fishes of Tennessee. University of Tennessee Press, Knoxville. 689 pp.

Hansen, K.A., B.K. Jones, M. Laha, and H.T. Mattingly. 2006. Population status and nesting biology of the rare Barrens Darter, *Etheostoma forbesi*. The American Midland Naturalist 155:63-69.

Martin, A.D., G.A. Zapfe, and H.T. Mattingly. 1999. Abundance of the Brook Darter, *Etheostoma burri*, in selected Black River tributaries. Journal of Freshwater Ecology 14:141-148.

McMahon, T.E., A.V. Zale, and D.J. Orth. 1996. Aquatic habitat measurements, pp. 83-120 in: B.R. Murphy and D.W. Willis (eds.). Fisheries techniques 2nd ed. American Fisheries Society, Bethesda, Maryland.

Osborne, L.L. and M.J. Wiley. 1992. Influence of tributary spatial position on the structure of warmwater fish communities. Canadian Journal of Fisheries and Aquatic Sciences 49:671-681.

Page, L.M. 1980. The life histories of *Etheostoma olivaceum* and *Etheostoma striatulum*, two species of darters in central Tennessee. Illinois Natural History Survey Biological Note 113:3-14.

Page, L.M. and M.E. Braasch. 1977. Systematic studies of darters of the subgenus *Catonotus* (Percidae), with the description of a new species from the Duck River System. Occasional Papers of the Museum of Natural History University of Kansas 63:1-18.

Page, L.M. and D.W. Schemske. 1978. The effect of interspecific competition on the distribution and size of darters of the subgenus *Catonotus* (Percidae: *Etheostoma*). Copeia 1978:406-412.

Page, L.M., P.A. Ceas, D.L. Swofford, and D.G. Buth. 1992. Evolutionary relationships within the *Etheostoma squamiceps* complex (Percidae; subgenus *Catonotus*) with descriptions of five new species. Copeia 1992:615-646.

SAS Institute, Inc. 1995. Statistical Analysis System 8.2. SAS Institute, Inc., Cary, North Carolina.

Strange, R.M. 2000. Evolution of sexual dimorphisms in spottail darters (Teleostei: Percidae). Ph.D. Dissertation, University of Alabama, Tuscaloosa. 126 pp.

Warren, M.L., B.M. Burr, and C.A. Taylor. 1994. The Relict Darter, *Etheostoma chienense* (Percidae): status review of a Kentucky endemic. Transactions of the Kentucky Academy of Science 55:20-27.

Werner, E.E. and J.F. Gilliam. 1984. The ontogenetic niche and species interactions in size-structured populations. Annual Review of Ecology and Systematics 15:393-425.

TABLE 1. Number of individuals of *Etheostoma striatulum*, *E. flabellare*, and *E. crossopterum* + *E. nigripinne* observed at 30 sites sampled during June–July 2006 in the Duck River system of middle Tennessee. Site locations are illustrated in Fig. 2.

			Number of Individuals Observed			
			E. striatulum			
Site	Stream	County	Age 0	Age 1+	*E. flabellare*	*E. crossopterum* + *E. nigripinne*
1	Wartrace Creek	Bedford	0	0	0	319
2	Flat Creek	Bedford	8	0	0	22
3	Hurricane Creek	Bedford	0	3	1	19
4	Fall Creek	Bedford	7	2	1	6
5	North Fork Creek	Bedford	0	3	0	60
6	Wilson Creek	Marshall	0	0	0	59
7	Duck River	Marshall	0	0	2	1
8	East Rock Creek	Marshall	0	0	0	0
9	Flat Creek	Maury	14	2	0	53
10	West Fork Bigby Creek	Lewis	0	1	8	35
11	Butler Creek	Bedford	0	0	17	303
12	Dog Branch	Maury	0	0	0	28
13	Flat Creek	Maury	42	2	0	297
14	North Fork Creek	Bedford	0	4	0	43
15	Wartrace Creek	Bedford	5	6	0	7
16	Globe Creek	Maury	0	0	0	0
17	Little Bigby Creek	Maury	0	0	0	1
18	West Fork Bigby Creek	Maury	0	0	0	1
19	East Rock Creek	Marshall	0	0	0	81
20	Little Flat Creek	Maury	0	0	0	67
21	Clem Creek	Bedford	0	0	0	13
22	Alexander Creek	Bedford	2	0	0	33
23	Flat Creek	Bedford	0	0	6	10
24	Knob Creek	Maury	0	0	3	1
25	Silver Creek	Maury	0	0	7	74
26	Fountain Creek	Maury	0	0	2	0
27	Noah Fork	Coffee	0	1	0	0
28	Garrison Fork	Bedford	0	0	0	0
29	Big Bigby Creek	Maury	0	0	12	21
30	Noah Fork	Coffee	0	0	7	2
		Totals	78	24	66	1,556

TABLE 2. Descriptive statistics and Fisher's Exact Test results (*P* value in rightmost column) for 12 environmental characteristics measured at 30 sites in the middle-upper Duck River system where *Etheostoma striatulum* was present (N = 11) or absent (N = 9) during a survey in June-July 2006.

	Striated Darter Present Sites			Striated Darter Absent Sites			
Characteristic	N	Mean ± SD	Range	N	Mean ± SD	Range	*P*
Elevation (m above sea level)	11	215 ± 18	186-250	19	212 ± 24	177-256	0.414
Stream Order	11	4.3 ± 0.7	3-5	18	4.0 ± 0.8	2-5	0.906
Link Magnitude	11	65 ± 32	27-129	18	74 ± 72	2-264	0.841
Riparian Zone Width (m)	11	11 ± 7	5-20	19	12 ± 9	0-30	0.319
Wetted Channel Width (m)	11	11 ± 4	5-19	19	14 ± 15	6-75	0.767
Mid-channel Depth (cm)	5	25 ± 20	9-44	11	27 ± 15	7-60	1.000
Discharge (m^3/s)	6	0.01 ± 0.01	0.0-0.01	12	0.42 ± 1.38	0.00-4.79	1.000
Water Temperature (C)	11	26 ± 2	21-28	19	26 ± 3	21-30	0.202
pH	3	8.0 ± 0.0	7.9-8.3	9	7.9 ± 0.4	7.6-8.6	0.763
Conductivity (□S)	7	247 ± 108	152-376	17	238 ± 116	27-391	0.261
Available Microhabitat	11	1.5 ± 0.8	0.2-3.0	19	1.4 ± 0.7	0.0-3.0	0.784
Abundance of other *Catonotus*	11	53 ± 83	0-297	19	51 ± 96	0-319	0.520

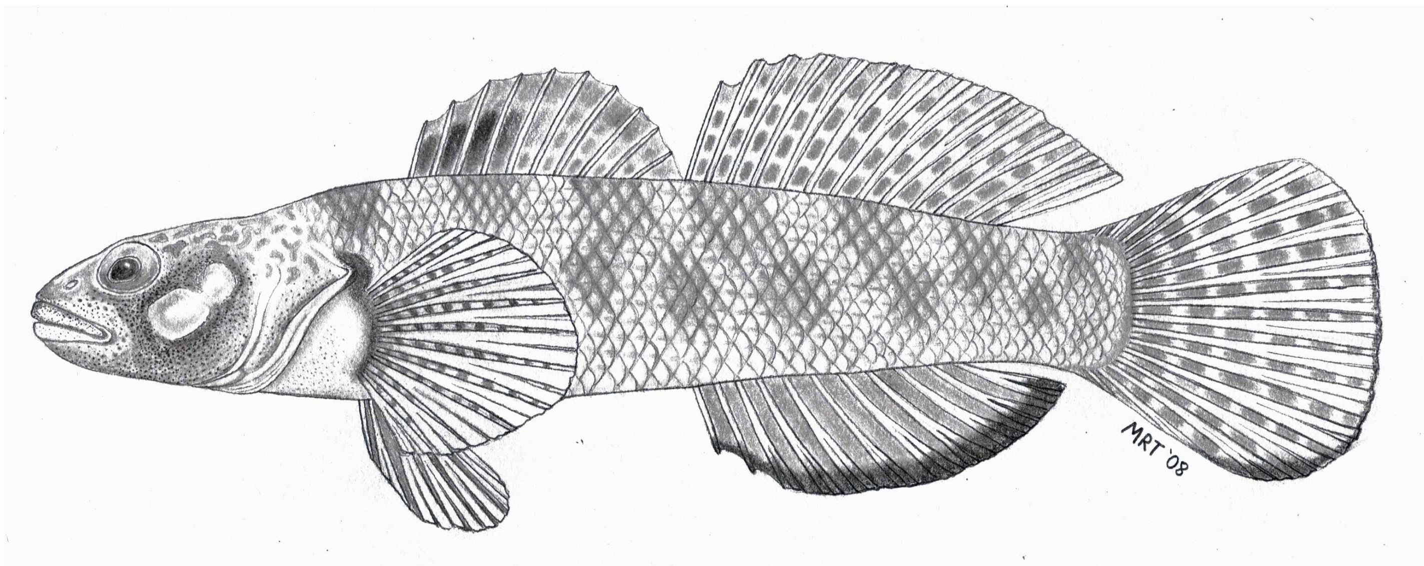


FIGURE 1. The Striated Darter, *Etheostoma striatulum*, as illustrated by Matthew R. Thomas.

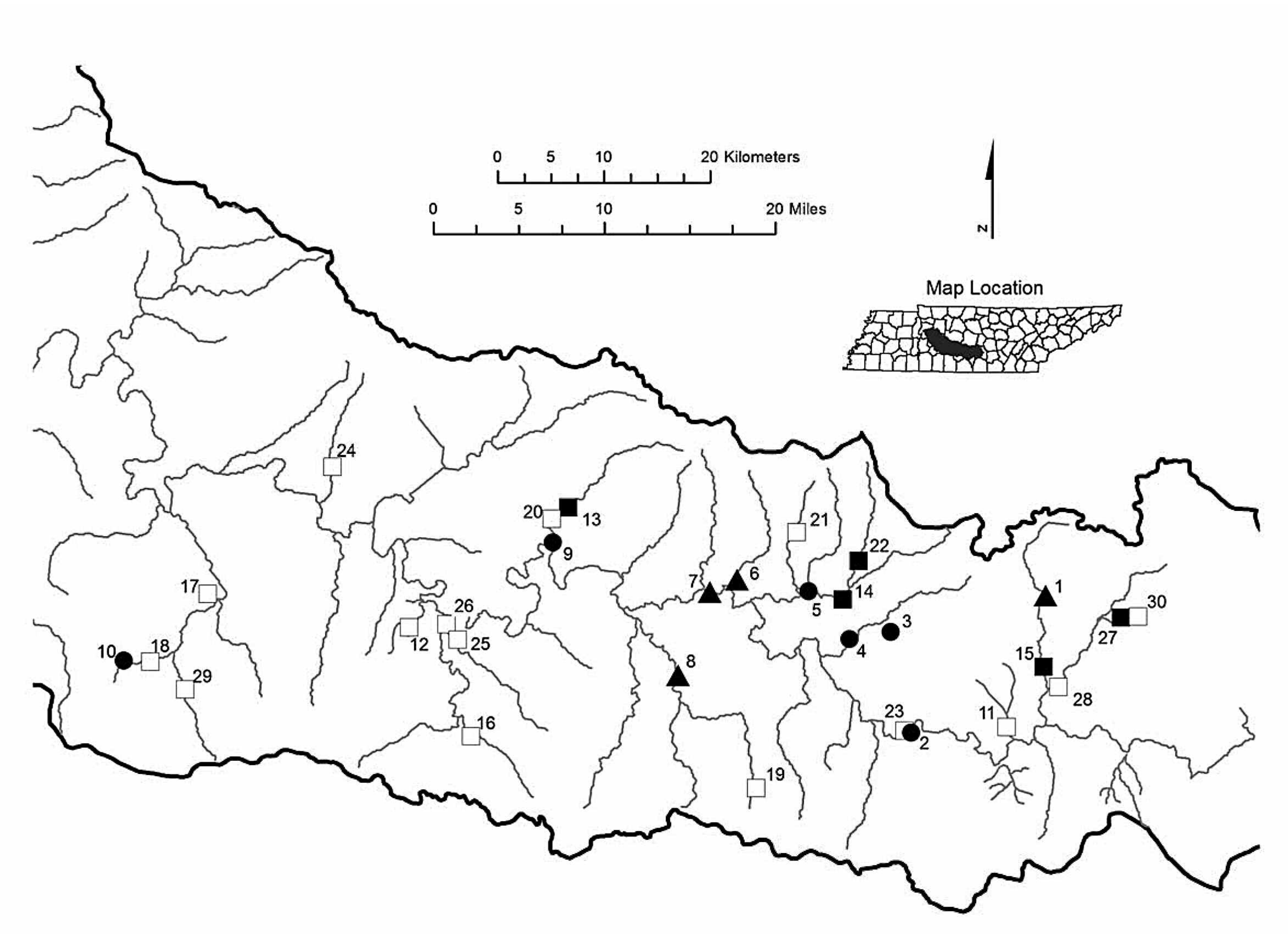


FIGURE 2. Middle-upper portion of the Duck River drainage in Tennessee showing 30 sites sampled during June-July 2006 for the Striated Darter, *Etheostoma striatulum*. Site coordinates can be found in Abernathy (2007). Filled circles indicate Striated Darter presence in both 2006 and a 1992 survey by Cook et al. (1996); filled triangles indicate absence in 2006 and presence in 1992; filled squares indicate presence in 2006 and absence or not sampled in 1992; and empty squares indicate absence in 2006 and not sampled in 1992.

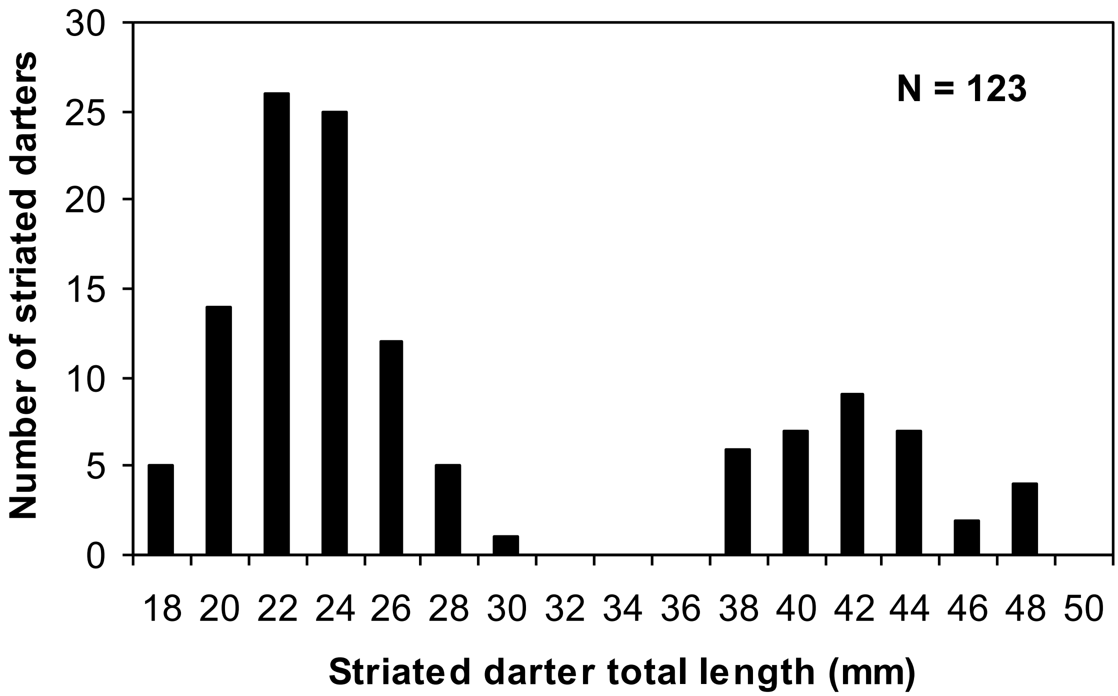


FIGURE 3. Length-frequency histogram depicting Striated Darter age-class structure at population estimate study site (2) and pilot study sites (13, 15) on 24-26 July 2006. Length intervals from left are 18-19 mm TL, 20-21 mm TL, and so forth.

Conservation Status of the Longhead Darter, *Percina macrocephala*, in Kinniconick Creek, Kentucky

DAVID A. EISENHOUR[1*], AUDREY M. RICHTER[1,2], and JOSHUA M. SCHIERING[1]

[1]Department of Biology and Chemistry
Morehead State University
Morehead, Kentucky 40351

[2]Present address:
U.S. Army Corps of Engineers, Buffalo District
Buffalo, New York 14207

**Corresponding author*: d.eisenhour@morehead-st.edu

ABSTRACT

Percina macrocephala, the Longhead Darter, is rare throughout its range and endangered in Kentucky. One population in Kentucky occurs in Kinniconick Creek, Lewis County, and prior to this study was known from only a few specimens, mostly collected in 1981. In summer and early fall of 2007 and 2008, 55 reaches, spanning 54 stream km of Kinniconick Creek, were surveyed by snorkeling, electrofishing, or seining for *P. macrocephala*. We encountered 104 individuals of *P. macrocephala* in a 50 stream km segment from just below the town of Kinniconick to the town of Garrison. Fifteen of the 55 sampled reaches contained *P. macrocephala*. Most individuals were encountered in a middle section between the confluences of Laurel Fork and Town Branch; this area also contained many young-of-the-year, indicating successful reproduction and recruitment. Although we judge this species to be rare to uncommon in most of Kinniconick Creek, it is locally common in the middle section, and the population seems to be stable and perhaps the most robust in the state. We conservatively estimate a total population of 2000-5000 in the stream. Because this population appears to be migratory, and exhibits source-sink dynamics, it is susceptible to anthropogenic barriers (e.g., culvert crossings) that prevent movements.

INTRODUCTION

Percina macrocephala (Cope), the Longhead Darter, is a large darter found in small to medium upland streams within the Ohio River basin (Page and Burr, 2011). However, it is sporadically distributed; the best populations appear to be in the Allegheny River drainage of Pennsylvania (D. A. Neely, pers. comm.), the Elk River of West Virginia (Stauffer et al., 1995; Welsh and Perry, 1998), and the Green River drainage in Kentucky and Tennessee (Page, 1978; Burr and Warren, 1986). Because of its discontinuous distribution and its rarity in many areas of occurrence, it is considered threatened or endangered in most states in its range; in Ohio it is probably extirpated (Trautman, 1981; NatureServe, 2010). In the southeastern U.S. it is considered threatened (Jelks et al., 2008) and globally is considered as G3 (vulnerable) (NatureServe, 2010).

In Kentucky, *P. macrocephala* is most common in the upper Green River and Barren River systems (Burr and Warren, 1986). One early record each is available from the Cumberland River drainage (1891) and the upper Kentucky River drainage (1890) (Kirsch, 1893; Page, 1978), but the species is now considered extirpated from these drainages (Burr and Warren, 1986). It is represented in the Big Sandy River system by a 1937 record from Johns Creek in Pike County (UMMZ 154793). Its current status there is unknown. *Percina macrocephala* was first documented in Kinniconick Creek by a specimen (SIUC 23370) collected by Minor Clark in 1938 and later by seven specimens collected by L. Kornman in 1981 from three sites (Warren and Cicerello, 1983). A few additional specimens were observed or captured from one of Kornman's collection sites from 2003-2005 (R. Cicerello and D. Neely, pers. comm.). Despite Warren and Cicerello's statement that Kinniconick Creek had a healthy population, no comprehensive survey for this species had been conducted prior to this survey. In Kentucky this species is listed as endangered (KSNPC, 2010) and critically imperiled (S1) (Kentucky's Comprehensive Wildlife Conservation Strategy, 2010).

Geographic variation in morphology has been noted in this species; the Kinniconick population is the only extant one in the state belonging to an upper Ohio group (Page, 1978). However, recent analysis of molecular data shows little differentiation among specimens from the Barren River to the Allegheny River, suggesting recent, post-

Pleistocene dispersal (Page and Near, 2007). Thus, a conservation survey provides information on one of the most poorly known extant populations in Kentucky. Our goals were to determine the distribution and population size of *P. macrocephala* in Kinniconick Creek. These data are compared with historical data and observed habitat to determine changes in population size and primary threats to this species in Kinniconick Creek.

METHODS

Study area

The Kinniconick Creek watershed (655 km^2) encompasses approximately half of Lewis County, Kentucky (USGS, 2008). This creek is 87 km long, with a gradient of approximately 2.3 m per stream km. The dominant land-cover type of the Kinniconick Creek watershed was calculated to be forested land (82%), followed by scrubby/herbaceous cover (7%), pasture/hay/crops (6%), and developed land (4%) (KGSGL, 2007). In general water is fairly clear (our measured Secchi disk visibilities were usually 2-3 m), with substrates primarily of boulder, cobble, and gravel. Submergent vegetation is occasional and emergent vegetation (*Justicia*) is common.

Sampling stations

We surveyed Kinniconick Creek, Lewis County, Kentucky from early summer to mid-fall of 2007 and 2008 for *P. macrocephala*. We established 198 sampling stations (reaches), via canoe, over a 69 stream km distance, from the confluence of Indian Creek to the town of Garrison (at the most downstream riffle before the stream enters the Ohio River). Reaches were defined as the crest of one riffle to the crest of the next riffle, and contained at least one pool. Reach length ranged from 36 to 3000 m, reach width averaged 13.4 m, and reach depth averaged 0.36 m. Because of logistical constraints and results from preliminary sampling, we sampled for darters in the lower 54 stream km (155 reaches), with the upper boundary being just above a concrete low-water farm bridge at the town of Kinniconick. We followed a stratified random design (Brown and Austen, 1996), where one of every three reaches were chosen randomly and surveyed for *P. macrocephala*. We snorkeled a total of 55 reaches (41 in 2007, 14 in 2008). In addition, 14 of these 55 reaches (selected randomly) were also sampled by backpack electrofishing and seining to evaluate the effectiveness of snorkeling.

Darter surveys

Quantitative surveys for darters were conducted by snorkeling and were accomplished by two persons moving parallel upstream through a reach. Positions of observed *P. macrocephala* were marked with a weighted flag. If multiple darters were found in a small area (< 1 m^2), only one flag was dropped but the maximum number of darters visible together was recorded. For reaches less than 120 m long, the entire reach was sampled. Longer reaches were subsampled by snorkeling 40 m at each end of the reach and 40 m near the middle of the reach. An additional criterion for the middle 40-m section was that it had to be sufficiently shallow (less than 1.5 m) to effectively snorkel, seine, and backpack electrofish. For reaches that also were sampled by backpack electrofishing and seining, the same distances were sampled. Sampling methodology of seining and electrofishing followed guidelines of KDOW (2010). Locations of darters observed while wading or canoeing in reaches or sections of reaches not sampled also were recorded.

RESULTS

We found *P. macrocephala* in 15 of the 55 reaches sampled (Fig. 1). Visibility (lateral Secchi disk distance) was not significantly different (t-test, P=0.1851) between reaches with and without *P. macrocephala*, suggesting differences in visibility among reaches did not affect our ability to detect darters while snorkeling. Most Longhead Darters were found in the middle part of Kinniconick Creek, between the confluences of Laurel Fork and Town Branch. A total of 104 *P. macrocephala* were encountered, which included 65 individuals from sampled reaches and 39 additional individuals (Appendix). These 39 individuals were observed, often from canoe or while wading, in reaches not sampled or in portions of reaches not sampled. Also, in 2005-2007, R. Cicerello and R. Evans, while surveying Kinniconick Creek for mussels, observed *P. macrocephala* at four sites (pers. comm.) and in 2003-2004, D. Neely collected a total of four *P. macrocephala* (pers. comm.) (Fig. 1). Our records extend the known range upstream and downstream in Kinniconick Creek to 50 stream km, from about 0.5 km below KY 10 Crossing (38.59782°, -83.18539°) upstream to about 3 km below confluence of Grassy Branch (38.50764°, -83.32468°). Both young-of-the-year and subadults-adults were found; all of the young-of-the-year were found below the confluence of Laurel Creek (Fig. 2).

Longhead Darters most frequently were found in areas just above riffles where there was little or no flow (0-0.22 m/sec, mean = 0.027 m/sec), low to moderate silt (<1 mm on rocks), abundant boulders and cobbles, and depths of 0.4-0.8 m. We occasionally encountered *P. macrocephala* below riffles and rarely encountered them in the middle of long pools, usually when shallow water (a "saddle") created slight flow (Eisenhour et al., 2009).

DISCUSSION

Status in Kinniconick Creek

Prior to 2003, *P. macrocephala* was known from only eight specimens, mostly collected by L. Kornman in 1981 (Warren and Cicerello, 1983) from a 25 stream km reach. We document the species from about 50 stream km and found it to be locally common in some areas. Because of the difficulty in capturing *P. macrocephala* in Kinniconick

Creek, mark-and-recapture studies are not useful for estimating populations. Based on a combination of snorkeling surveys and mark-and-recapture methods, a population of Gilt Darters (*Percina evides*) in Tellico Creek, North Carolina, was estimated to be about 2.7 times larger than the number of individuals encountered (Skyfield and Grossman, 2008). Our snorkeling efforts appear a little less intense than theirs, but we judge that *P. macrocephala* is more likely to be observed, because of its large size and pelagic habits. Assuming the detection probability of *P. evides* and *P. macrocephala* by snorkeling is similar, we estimate 20-50% of the individuals present were seen in the sections sampled. Extrapolating from the 5700 m sampled to the 54 total km in our survey area, a conservative population estimate for Kinniconick Creek is 2000-5000 *P. macrocephala*. There is no evidence to suggest that *P. macrocephala* has declined in Kinniconick Creek. We judge *P. macrocephala* to be uncommon to locally common in Kinniconick Creek below the confluence of Laurel Fork and rare above the confluence of Laurel Fork.

The population of *P. macrocephala* in Kinniconick Creek may be one of the most robust in the state. This species formerly was common in the upper Green and Barren River systems (Page, 1978), and remains locally common there in Russell Creek, Trammel Fork, and Drakes Creek (D. A. Neely, pers. comm.). Other recent, intensive surveys of areas in the Green River where it was formerly common revealed very few specimens (M. Thomas, B. M. Burr, and R. Hopkins, pers. comm.), suggesting some populations in the Green River drainage have declined. Although this study documented a Kinniconick population larger than previously presumed and relatively stable, *P. macrocephala* is still uncommon there, and one of the rarest darters in Kinniconick Creek. We recommend maintaining the endangered status of this species in Kentucky.

Management implications

1. Snorkeling is an effective sampling technique. Most Longhead Darters were seen while snorkeling (74%), many were seen while canoeing or wading (20%), but only a few were captured by electrofishing or seining (6%). These darters are large and typically suspend in midwater, making them fairly easy to see by snorkeling or from the surface. In many reaches we saw numerous *P. macrocephala* while snorkeling, but were unable to collect any with a seine or backpack electrofisher only minutes later. We sometimes observed *P. macrocephala* moving away from an active backpack electrofisher, apparently sensing the electrical field from a distance and escaping before they could be stunned, as do pelagic minnows.

2. Priority protection. The most important section of Kinniconick Creek that should be protected is the middle section, between the confluences of Laurel Fork and Town Branch. This area has the highest concentrations of *P. macrocephala*, and nearly all of the young-of-the-year. In addition, this is the only section where we encountered another rare fish (*Notropis ariommus*, Popeye Shiner), and where we most frequently observed live mussels. Riparian zones along this section are almost entirely intact and land cover is almost entirely forested. Our preliminary observations suggest a study comparing the relationship of *P. macrocephala* abundance to land use practices and riparian zone width would likely be informative.

3. Road crossings should allow instream movements of Longhead Darters. Populations appear to be seasonally migratory. At two sites that each yielded more than 10 individuals during the regular sampling protocol (August 2007), no individuals were found at two other surveys (June 2007 and September 2009), despite similar, intensive efforts. Kinniconick Creek experienced severe drought both sampling seasons, with record to near-record low flows recorded at the USGS gaging station. Flows fell to zero, or nearly so, by mid-July in 2007 and by late August in 2008. At this time, lower Kinniconick Creek (below confluence of Laurel Fork) flow was mainly interstitial seepage through riffles; upper Kinniconick Creek consisted of isolated pools, with intervening, completely dewatered sections as long as 500 m. We suspect that periodic local extirpations or poor recruitment occurs in the upper portion of the stream during severe droughts; immigration from downstream areas is needed for recolonization. Phylogeographic studies support the hypothesis that this species has substantial potential for migration. Specimens spanning a geographic range from the Barren River of Kentucky to the Allegheny River of Pennsylvania exhibit almost no divergence in mtDNA, which suggests a recent population bottleneck followed by rapid, postglacial dispersal (Page and Near, 2007). In addition, the distribution of adult and young *P. macrocephala* (Fig. 2) suggest that source-sink dynamics (Pulliam, 1988; Dias, 1996) are present, at least during our sample period, which were both severe drought years. Downstream areas which have a high density of *P. macrocephala* and evidence of successful reproduction may act as source populations that supplement populations, by migration, in upper Kinniconick Creek, a sink. The upper Kinniconick populations may require immigration to supplement limited reproduction (acting as a pseudosink) or no reproduction (acting as a true sink).

Some road crossings, especially culverts, affect fish movements by blocking or limiting migration (Warren and Pardew, 1998; Schaefer et al., 2003; Benton et al., 2008). Many road crossings over Kinniconick Creek are high bridges, such as those at KY 10 and KY 59, which do not impair fish movement. However, two crossings at the upstream limit of the range of *P. macrocephala* in Kinniconick Creek are low-water concrete bridges with perched culverts. Upstream passage by fishes is impossible at low to moderate flows, and difficult at high flow, because flow is funneled through culverts, creating rapid currents with no cover. Sampling above the most downstream culvert was less intensive (five sites quantitatively sampled, plus about 12 km of survey by canoe and wading), than in the rest of the stream, but no *P. macrocephala* were found in this area. This suggests that construction of road crossings be engineered to permit upstream and downstream movement of fishes.

ACKNOWLEDGMENTS

This project was inspired by discussions with Lew Kornman (Kentucky Department of Fish and Wildlife Resources), who rediscovered *P. macrocephala* in Kinniconick Creek in the 1980s. We wish to thank the following individuals for assisting in fieldwork: R. Broadway, J. D. Eisenhour, L. V. Eisenhour, T. Evans, A. J. Farwick, S. Fryman, J. Taylor, M. Thomas, and J. Thompson. R. Cicerello, R. Evans, and D. Neely provided records of *P. macrocephala* observed during and just prior to our survey. Funding was provided by the State Wildlife Grants Program, administered via KDFWR. Permits to work with fishes were provided by KDFWR and Morehead State University IACUC.

LITERATURE CITED

Benton, P. D., W. E. Ensign, and B. J. Freeman. 2008. The effect of road crossings on fish movements in small Etowah Basin streams. Southeast. Nat. 7:301-310.

Brown, M. L., and D. J. Austen. 1996. Data management and statistical techniques, pp. 17-62. *In:* B. R. Murphy and D. W. Willis, eds., Fisheries Techniques, 2nd ed. Amer. Fish. Soc., Bethesda, Maryland.

Burr, B. M., and M. L. Warren, Jr. 1986. A distributional atlas of Kentucky fishes. Kentucky Nat. Preserves Comm., Sci. Tech. Series 4:1-398.

Dias, P. C. 1996. Sources and sinks in population biology. Trends Ecol. Evol. 11:326-330.

Eisenhour, D. J., J. M. Schiering, and A. M. Richter. 2009. Conservation status and habitat of the longhead darter, *Percina macrocephala*, in Kinniconick Creek, Kentucky. Final Report to Kentucky Department of Fish and Wildlife Resources. 40 pp.

Jelks, H. L., S. J. Walsh, N. M. Burkhead, S. Contreras-Balderas, E. Diaz-Pardo, D. A. Hendrickson, J. Lyons, N. E. Mandrak, F. McCormick, J. S. Nelson, S. P. Platania, B. A. Porter, C. B. Renaud, J. J. Schmitter-Soto, E. B. Taylor, and M. L. Warren. 2008. Endangered species: Conservation status of imperiled North American freshwater and diadromous fishes. Fisheries 33:372-407.

Kentucky's Comprehensive Wildlife Conservation Strategy. 2010. Kentucky Department of Fish and Wildlife Resources, Kentucky 40601. http://fw.ky.gov/kfwis/stwg/ (Updated 7 Dec 2010).

Kentucky Division of Water (KDOW). 2010. Collection methods for fishes in wadeable streams, version 2.1. Kentucky Department for Environmental Protection, Division of Water, Frankfort, Kentucky.

Kentucky Geological Survey Geospatial Library (KGSGL) 2007. http://www.uky.ed/KGS/gis_gis.html (Accessed October 13 2007).

Kentucky State Nature Preserves Commission (KSNPC). 2010. Rare and extirpated biota and natural communities of Kentucky. J. Kentucky Acad. Sci. 71:67-81.

Kirsch, P. H. 1893. Notes on collection of fishes from the southern tributaries of the Cumberland River in Kentucky and Tennessee. Bull. U.S. Fish Comm. 11:257-265.

NatureServe. 2010. NatureServe Explorer: An online encyclopedia of life [web application]. Version 7.1. NatureServe, Arlington, Virginia. Available http://www.natureserve.org/explorer (Updated August 2010).

Page, L. M. 1978. Redescription, distribution, variation and life history notes on *Percina macrocephala* (Percidae). Copeia 1978:655-664.

Page, L. M., and B. M. Burr. 2011. Peterson field guide to freshwater fishes, 2nd ed. Houghton Mifflin Harcourt, Boston, MA.

Page, L. M., and T. J. Near. 2007. A new darter from the Upper Tennessee River drainage related to *Percina macrocephala* (Percidae: Etheostomatinae). Copeia 2007:605-613.

Pulliam, H. R. 1988. Sources, sinks, and population regulation. Amer. Nat. 132:652-661.

Schaefer, J. F., E. Marsh-Matthews, D. E. Spooner, K. B. Gido, and W. J. Matthews. 2003. Effects of barriers and thermal refugia on local movement of the threatened leopard darter, *Percina pantherina*, Environ. Biol. Fishes. 66:391-400.

Skyfield, J. P., and G. D. Grossman. 2008. Microhabitats, movements and habitat use by gilt darters (*Percina evides*) in southern Appalachian (USA) streams. Ecol. Freshw. Fish. 17:219-230.

Stauffer, J. R., Jr., J. M. Boltz, and L. R. White. 1995. Fishes of West Virginia. Proc. Acad. Nat. Sci., Philadelphia 146:1-389.

Trautman, M. B. 1981. Fishes of Ohio, 2nd ed. Ohio State Univ. Press, Columbus.

USGS National Water Information System. 2008. http://waterdata.usgs.gov (Accessed 9 February 2008).

Warren, M. L., Jr., and R. R. Cicerello. 1983. Drainage records and conservation status evaluations for thirteen Kentucky fishes. Brimleyana 9:97-109.

Warren, M. L., Jr., and M. G. Pardew. 1998. Road crossings as barriers to small-stream fish movement. Trans. Amer. Fish. Soc. 127:637-644.

Welsh, S. A., and S. A. Perry. 1998. Habitat partitioning in a community of darters in the Elk River, West Virginia. Environ. Biol. Fishes. 51:411-419.

APPENDIX

TABLE A1. *Percina macrocephala* found within quantitatively sampled reaches in Kinniconick Creek. Reaches are listed from upstream to downstream.

Date	Reach	# of YOY	# of adults	Method	Latitude and Longitude
13 October 2007	50		1	Snorkel	38.50764°, -83.32468°
			1	Seine	
23 September 2007	91		2	Snorkel	38.48405°, -83.27621°
			1	Shock	
14 August 2007	104		1	Snorkel	38.48222°, -83.25028°
22 June 2007	106		1	Snorkel	38.48444°, -83.24694°
			1	Shock	
25 July 2007	112	12		Snorkel	38.49722°, -83.25637°
24 July 2007	123	6	2	Snorkel	38.52222°, -83.24861°
24 July 2007	125		3	Snorkel	38.52278°, -83.24986°
31 August 2007	132		2	Snorkel	38.51579°, -83.23318°
9 September 2007	138	2	4	Snorkel	38.53078°, -83.24277°
31 August 2007	140	1	6	Snorkel	38.53401°, -83.23682°
			7	Snorkel	38.53376°, -83.23737°
16 August 2007	143	4		Snorkel	38.54194°, -83.23806°
8 Aug 2007	148		1	Snorkel	38.55066°, -83.23826°
30 July 2008	K23	2		Snorkel	38.57521°, -83.19028°
			1	Snorkel	38.5726°, -83.19028°
			1	Snorkel	38.57509°, -83.19016°
8 August 2008	K34		1	Snorkel	38.58294°, -83.19058°
23 August 2008	K38	2		Snorkel	38.59782°, -83.18539°

TABLE A2. *Percina macrocephala* found during non-quantitative surveys (either in reaches not sampled or in parts of reaches not quantitatively sampled) in Kinniconick Creek. Reaches are listed from upstream to downstream.

Date	Reach	# of YOY	# of adults	Method	Latitude and Longitude
31 May 2007	106		1	Canoe	38.48417°, -83.2544°
			2	Snorkel	
31 May 2007	106		1	Canoe	38.48472°, -83.25361°
25 July 2007	109	1		Wading	38.49171°, -83.25097°
25 July 2007	110		2	Canoe	38.49444°, -83.25°
25 July 2007	111	1		Canoe	38.49667°, -83.25806°
25 July 2007	111	3		Canoe	38.49692°, -83.25802°
		3		Wading	38. 49707°, -83.25725°
23 July 2007	122		1	Wading	38.52155°, -83.24861°
24 July 2007	126	10	6	Wading	38.52333°, -83.24444°
1 August 2007	126	3		Wading	38.52333°, -83.2444°
		1		Shock	
			2	Shock	
			1	Wading	
31 August 2007	131	1		Canoe	38.51674°, -83. 23483°

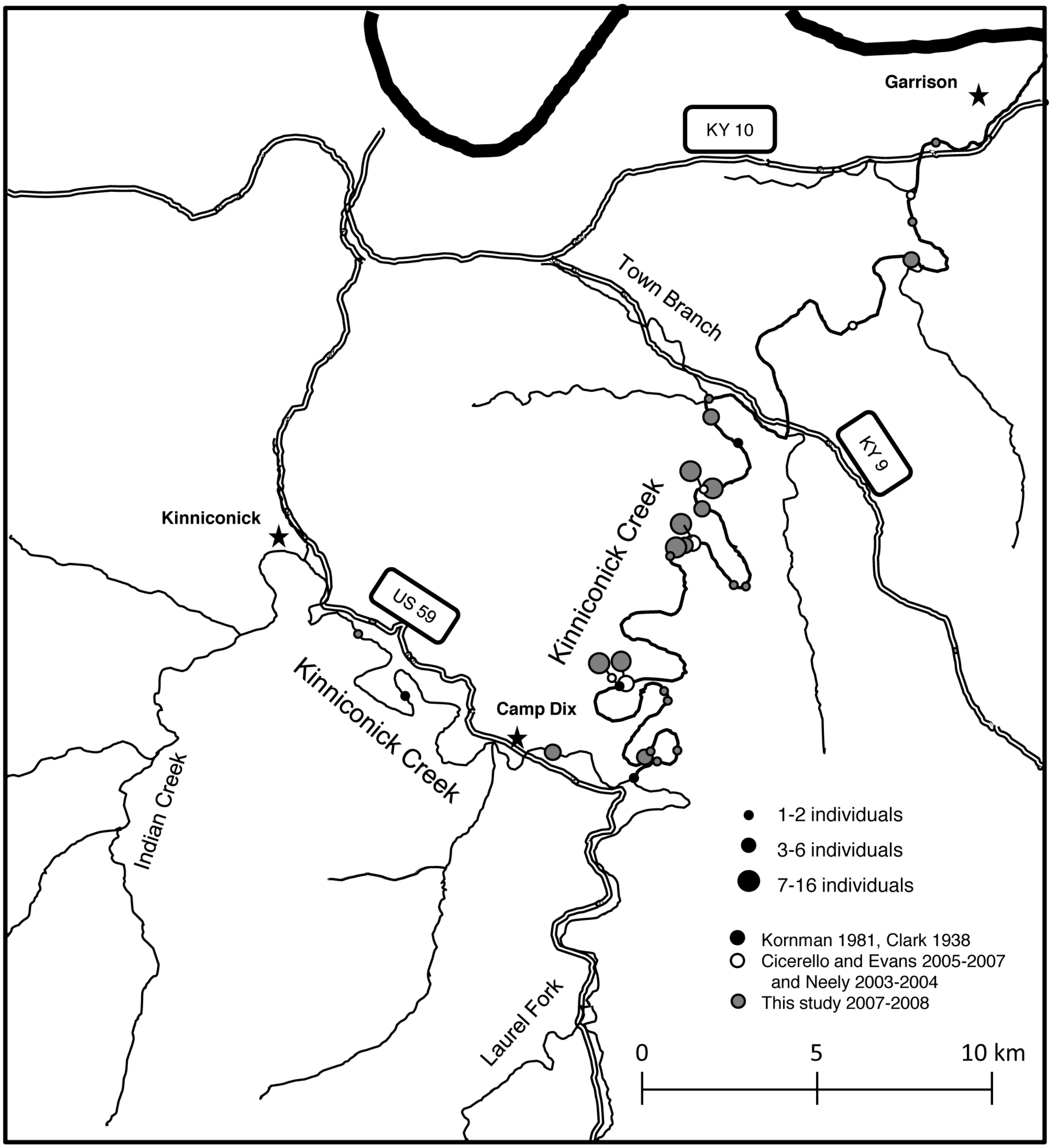


FIGURE 1. Distribution of *Percina macrocephala* in Kinniconick Creek, Lewis County, Kentucky.

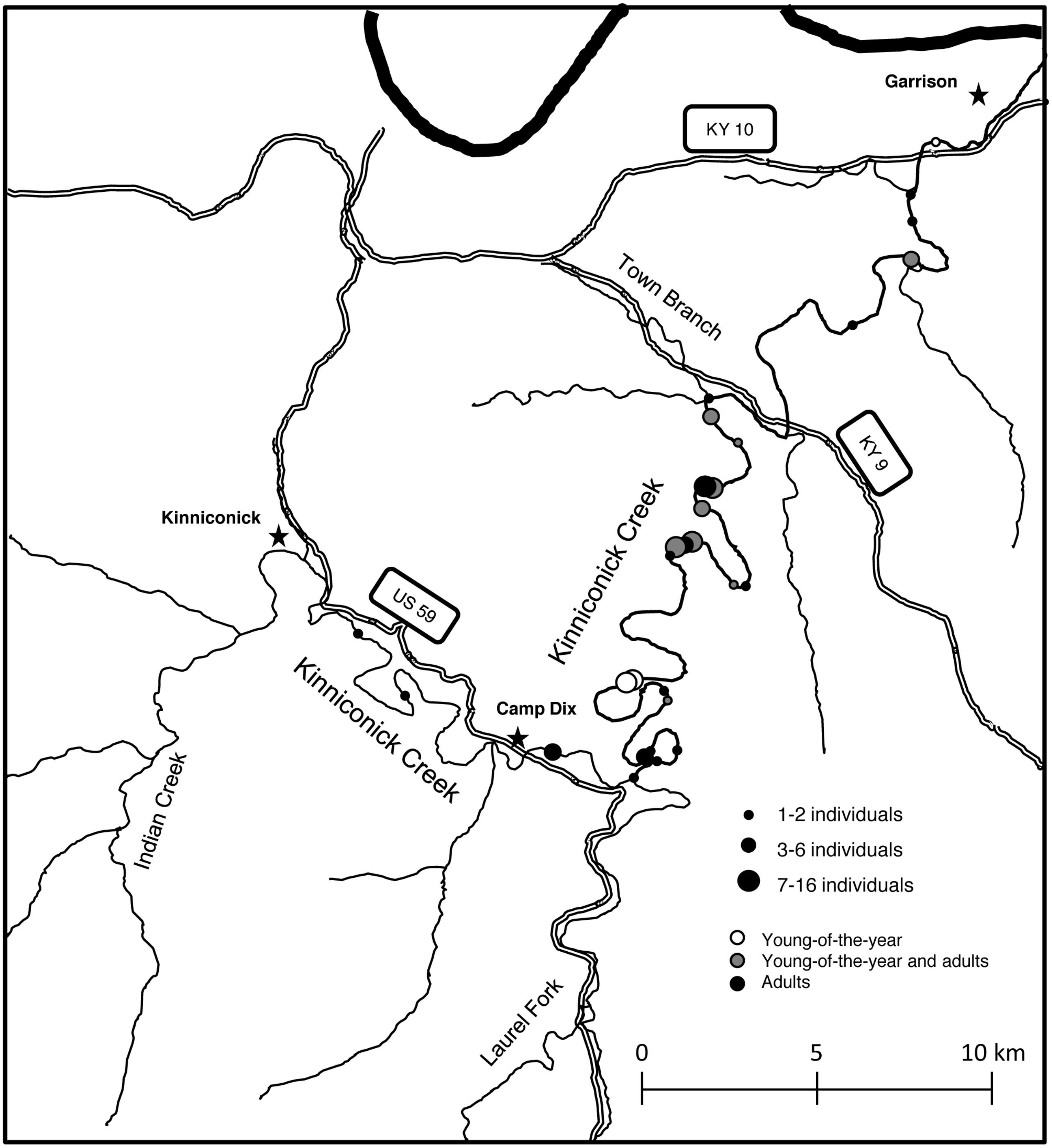


FIGURE 2. Adult and young *Percina macrocephala* encountered in Kinniconick Creek, 2007-2008.

First Observation of a Natural Hybrid Between Endangered Roanoke Logperch (*Percina rex*) and Chainback Darter (*Percina nevisense*)

JAMES H. ROBERTS

Department of Fisheries and Wildlife Sciences, Virginia Polytechnic Institute and State University, Blacksburg, Virginia, USA 24061; Email: darter@vt.edu

ABSTRACT

I used meristic, mitochondrial DNA, and nuclear DNA methods to infer the most likely ancestry of a putative hybrid specimen of *Percina* captured in the Roanoke River of Virginia. Potential parental species included *Percina rex*, *P. nevisense*, and *P. roanoka*. All nine of the meristic characters that I counted for the putative hybrid were within the published ranges of counts for *P. nevisense*, whereas counts for five characters were outside of published ranges for either *P. roanoka* or *P. rex*. These results were consistent with pure *P. nevisense* ancestry as well as various hybridization scenarios. Based on analysis of 1037 bp of the *ND2* mitochondrial gene, the haplotype of the putative hybrid was identical to a known *P. rex* haplotype, but <86% similar to the closest-matching haplotypes for either of the other two species. Bayesian admixture analysis using seven nuclear microsatellite markers indicated a high probability of *P. rex* or *P. nevisense* ancestry and a low probability of *P. roanoka* ancestry. Taking all evidence together, the most parsimonious explanation is that the specimen was a hybrid between *P. rex* and *P. nevisense*.

INTRODUCTION

Hybridization is a relatively common phenomenon among freshwater fishes (Scribner et al., 2001; Keck and Near, 2009). Although many hybrid offspring are not viable, those that are may contribute to subsequent introgression. Introgression can increase phylogenetic diversity via the creation of novel evolutionary trajectories (e.g., Dowling and Secor, 1997), but also can negatively impact native genomes and species (e.g., Echelle and Echelle, 1997; Seehausen et al., 1997). Documentation of hybridization events in nature therefore is important from both scientific and conservation standpoints.

On 16 July 2004, while using a backpack electrofisher to sample fishes in the Roanoke River (Roanoke County, Virginia), a montane warmwater stream in the Ridge and Valley physiographic province, I captured a putative hybrid of the genus *Percina*. The specimen exhibited pigmentation patterns unlike any other *Percina* species known from the Roanoke River drainage (Fig. 1; Jenkins and Burkhead, 1994). The right pectoral fin was removed for genetic analysis and both specimen and fin were preserved in 95% ethanol.

The only *Percina* species known to occur in the Roanoke drainage (Jenkins and Burkhead, 1994) are Roanoke Logperch, *Percina rex* (Jordan and Evermann); Chainback Darter, *P. nevisense* (Cope); and Roanoke Darter, *P. roanoka* (Jordan and Jenkins). All three species are syntopic in riffle-run habitats in the montane section of the Roanoke River, but the species vary greatly in abundance; *P. roanoka* is by far the most abundant, *P. rex* is intermediately abundant, and *P. nevisense* is by far the least abundant *Percina* species (J. Roberts, pers. obs.). Numerous darter hybrids involving *Percina* species have been observed previously in nature (Hocutt and Hambrick, 1973; Keck and Near, 2009). Of the three Roanoke River *Percina* species, hybridization has been observed only in *P. roanoka*, but there have been reported hybrids involving species closely related to the other two Roanoke River *Percina* species (e.g., *P. caprodes*, *P. peltata*). Furthermore, prezygotic reproductive isolating barriers (RIBs) for these species may be weak because of their preferences for similar spawning habitats and times, similar egg-burying strategies and modest sexual dimorphism. In the present study, I used meristic counts and molecular genetic markers to infer the most likely parental species of the putative hybrid individual.

METHODS

Meristic counts were made under a dissecting microscope on both sides of the specimen. Results were compared to published ranges of meristic counts from potential parental species, as follows: *P. rex* ranges were based on Jenkins and Burkhead's (1994) analysis of 112 Virginia collections, *P. roanoka* ranges were based on Jenkins and Burkhead's (1994) analysis of 80 Virginia collections combined with their synthesis of Mayden and Page's (1979) data, and *P. nevisense* ranges were based on Goodin et al. (1998) analysis of 115 collections from throughout the species' range.

To infer the matrilineal ancestry of the putative hybrid, I sampled 1037 bp of the *ND2* mitochondrial DNA gene. Whole genomic DNA was extracted from the fin clip using a Pure Gene DNA Extraction Core Kit A (Gentra Systems, Minneapolis, Minnesota, USA). Forward and reverse primers for polymerase chain reactions were *ND2* 562L and *ND2* 449H, respectively, from George et al. (2006). PCR employed 25-µl reactions with the following reagent mix: 2 µL of 2.5-mM each dNTPs (premixed); 2.5 µL of 10X NH_4 Ex*Taq* buffer ($MgCl_2$ included); 1 µL each of 20-µM ND2 562L and ND2 449H primers; 0.15 µL of 5 Units μL^{-1} Ex*Taq* polymerase (TaKaRa Bio, Inc., Otsu, Shiga, Japan); 3 µL of 20 ng μL^{-1} template DNA; and 15.35 µL of dH_2O. I conducted PCR in a MyCycler Thermal Cycler (BioRad, Hercules, California, USA) using an initial denaturation step (94 °C, 3 min), followed by 35 cycles of denaturation (94 °C, 40 s), annealing (60 °C, 40 s), and extension (72 °C, 60 s), and a final extension step (72 °C, 2 min). Non-specific amplification products were removed using ExoSAP-IT (USB Corp., Cleveland, Ohio, USA) and the cleaned DNA was subjected to forward and reverse sequencing in an ABI 3130 automated sequencer at the Virginia Bioinformatics Institute at Virginia Tech. Forward and reverse sequence fragments then were aligned and edited in SEQUENCHER version 3.0 (Gene Codes Corp., Ann Arbor, Michigan, USA). The resulting sequence was accessioned in the GenBank public database (Benson et al., 1999; accession number JF944898) and compared to published *ND2* sequences from potential parent species using a BLAST search of the database, conducted 23 May 2011.

I further examined the ancestry of the putative hybrid using nuclear DNA markers, which are biparentally inherited and recombinant, thus providing information on both parents. I analyzed the putative hybrid plus a suite of known-identity individuals from the three possible parent species (*i.e.*, 15 *P. rex*, 4 *P. nevisense*, and 5 *P. roanoka*) using 12 microsatellite markers (*Prex33*, *Prex34*, *Prex36*, *Prex37*, *Prex38*, *Prex41*, *Prex42*, *Prex43*, *Prex44*, *Prex45*, *Prex46* and *Prex47*) and conditions described by Dutton et al. (2008). Forward primers were labeled using one of the following four fluorescent dyes: NED, VIC, PET or FAM (Applied Biosystems, Inc., Foster City, California, USA). PCR was conducted in a MyCycler Thermal Cycler (BioRad, Hercules, California, USA). Amplification products were separated in an ABI 3130 automated sequencer at the Virginia Bioinformatics Institute at Virginia Tech and sized using GeneMapper version 3.5 and a LIZ500HD size standard (Applied Biosystems, Inc., Foster City, California, USA).

Only seven of the microsatellite markers (*Prex37*, *Prex38*, *Prex41*, *Prex43*, *Prex44*, *Prex46*, and *Prex47*) amplified reliably across all three potential parental species, presumably because of inter-specific mutations in microsatellite-flanking regions that prevented annealing of primers. I used data from these seven markers to infer the ancestry of the putative hybrid using two types of admixture analyses. First, NewHybrids 1.1 (Anderson, 2003) was used to estimate the Bayesian posterior probabilities that the putative hybrid belonged in each of six discrete hybrid categories (Anderson and Thompson, 2002). NewHybrids could accommodate only two parental species at a time, so two separate models were run. The first model estimated the probabilities that the putative hybrid was: 1) a pure *P. rex*, 2) a pure *P. nevisense*, 3) an F1 cross of these two species, 4) an F2 cross of two F1s, 5) an F1 x *P. rex* backcross, or 6) an F1 x *P. nevisense* backcross. In the second model, categories were similar except that *P. roanoka* was substituted for *P. nevisense*. I did not attempt to model the possibility of a *P. roanoka* x *P. nevisense* hybrid, given that mtDNA analysis indicated that *P. rex* was one of the ancestral species (see Results and Discussion). In both models, I used a Jeffreys-type prior distribution for the parental species' allele frequencies, as recommended by Anderson and Thompson (2002), and made an exhaustive set of 2.5 x 10^6 "sweeps" through the Markov-Chain-Monte-Carlo (MCMC) simulation algorithm.

The second admixture analysis employed STRUCTURE 2.1 (Pritchard et al., 2000) to estimate the probabilities of the putative hybrid's genome originating from each of the three possible parental species. The STRUCTURE model assumed three *a priori* genetic populations (i.e., $K = 3$) with independent allele frequencies, but allowed for potential background admixture and did not incorporate prior knowledge of individuals' species-identities. Parameter space was searched using 10^6 recorded MCMC chains, following a burn-in of 10^5 chains. To estimate the potential accuracy of admixture analyses for detecting hybrids, estimates of genetic differentiation (F_{ST}) were calculated in ARLEQUIN 3.11 (Excoffier et al., 2005) and compared to the simulation results of Vähä and Primmer (2006).

RESULTS AND DISCUSSION

All meristic counts were within meristic ranges previously observed for *P. nevisense*, but counts were not consistently within known ranges for the other two species (Fig. 2). Meristic counts on the putative hybrid for pectoral fin rays (13), anal fin spines (2) and anal fin rays (10) overlapped with the known meristic ranges of all three potential parent species. In contrast, the dorsal spine count (12) overlapped only with *P. roanoka* and *P. nevisense* and the count of scales above the lateral line (8) overlapped only with *P. rex* and *P. nevisense*. Remaining meristic counts for the putative hybrid, including dorsal fin rays (13), circum-caudal-peduncle scales (22), scales below the lateral line (11) and lateral line scales (63), overlapped with the range for *P. nevisense* only. Thus, meristic results were inconsistent with the hypotheses of pure *P. rex* or pure *P. roanoka* ancestry. However, because meristic characteristics of hybrid individuals may or may not be intermediate to parental species (e.g., Ross and Cavender, 1981), alternative hypotheses regarding meristics are possible, including: (1) pure *P. nevisense* ancestry, (2) meristic intermediacy in a *P. rex* x *P. roanoka* hybrid, or (3) meristic non-intermediacy in a hybrid cross involving *P. nevisense*.

Based on a BLAST search of the GenBank database, the haplotype of the putative hybrid was identical (1037 bp matching) to a published *P. rex ND2* mitochondrial DNA haplotype (accession number JF929012). The *P. rex* individual bearing this haplotype was captured in the upper Roanoke River (J. Roberts, unpublished data). In contrast, the closest-matching *P. roanoka ND2* haplotype (AY225722) was only 85% similar (883 of 1037 bp) to the hybrid haplotype. No *P. nevisense ND2* haplotypes were contained in the GenBank database, but the closest-matching *ND2* haplotype from *P. peltata* (AY770845), a close relative of *P. nevisense*, was only 84% similar (869 of 1033 bp) to the hybrid haplotype. Thus, there was strong evidence that one of the ancestral species was *P. rex*, though I could not conclude from this analysis, how far back in time the ancestry occurred (i.e., the hybrid individual could have been an F1, F2, backcross, etc.).

Analyses of nuclear DNA microsatellite data suggest that the most likely parental species of the putative hybrid were *P. rex* and *P. nevisense*. Estimates of F_{ST} were 0.13, 0.20, and 0.21 in pairwise comparisons of known-identity *P. rex* versus *P. roanoka*, *P. roanoka* versus *P. nevisense*, and *P. rex* versus *P. nevisense* specimens, respectively. Given this level of differentiation and the use of seven loci, STRUCTURE could detect hybrid ancestry ≥ 20% with estimated 60-80% accuracy, whereas NewHybrids could assign hybrid status with estimated 50-80% accuracy (Vähä and Primmer, 2006). In the NewHybrids model hypothesizing *P. rex* and/or *P. nevisense* as parental species, there was an essentially equal Bayesian posterior probability that the putative hybrid was a pure *P. rex* or a pure *P. nevisense* (P = 0.35) and the highest-probability hybrid category was F1 (P = 0.14) (Table 1). In the model hypothesizing *P. rex* and/or *P. roanoka* as parental species, there was a low probability for any category involving full or partial *P. roanoka* ancestry (P < 0.02), so the model assigned most of the probability to the pure *P. rex* category (P = 0.96). Both models performed well at classifying individuals of known identity to the correct species, with probabilities > 0.97 in all cases. NewHybrids thus indicated strong support for *P. rex* and *P. nevisense* ancestry, but weak support for *P. roanoka* ancestry. Difficulties teasing apart pure from hybrid ancestry may have stemmed from the somewhat low statistical power of the analysis, given only 7 loci (Vähä and Primmer, 2006).

The STRUCTURE analysis also performed well at assigning known-identity individuals to the correct species, but assignment of the putative hybrid was more ambiguous (Fig. 3). Assuming that the markers were not linked, we would expect an F1 hybrid to exhibit an approximately 0.5 probability of originating from each of two parental species. However, the Bayesian posterior probabilities of the putative hybrid being a *P. nevisense*, *P. roanoka*, or *P. rex* were 0.73, 0.17, and 0.09, respectively. Thus, STRUCTURE indicated strong support for *P. nevisense* ancestry, but weaker support for either other parent. Lack of strong support for *P. rex* ancestry suggests that the hybrid may have been a backcross with *P. nevisense*, though this hypothesis was not supported by the results of NewHybrids. Previous studies have revealed hybrid backcrosses of various other darter taxa that lacked strong nuclear introgression despite complete mitochondrial introgression (e.g., Bossu and Near, 2009; Keck and Near, 2009).

Given the preponderance of meristic and genetic evidence, the most parsimonious explanation is that the specimen was a hybrid between *P. rex* and *P. nevisense*. Analysis of mitochondrial DNA clearly indicated *P. rex* matrilineal ancestry, whereas nuclear DNA results were most consistent with admixture between *P. rex* and *P. nevisense*. Meristic data indicated either pure *P. nevisense* ancestry or hybridization, but could not be used to proffer one hybrid pairing over another. Although I cannot conclusively rule out the possibility of *P. roanoka* ancestry, this species was the least supported parental species across the analyses performed herein. Furthermore, data were inconclusive as to how many generations ago the hybridization event occurred, given that neither of the two admixture analyses clearly indicated that the hybrid was an F1.

No hybrids previously have been reported involving either *P. rex* or *P. nevisense*, yet this hybridization event is not especially surprising: both species are relatively large-bodied darters with similar ecological requirements and modest sexual dimorphism. Thus, prezygotic RIBs may be weak for these species. RIBs are known to break down following disturbances (Hubbs, 1955; Seehausen et al., 1997), though I am unaware of any novel environmental pressures that would increase hybridization rates in the Roanoke River. The prevalence and significance of such hybridization events are unknown. However, I presume that hybridization between *P. rex* and *P. nevisense* has been rare in the Roanoke River over the past 40 years, given that this was its first observation despite frequent surveys over this time period by workers from Virginia Tech and Roanoke College (Jenkins and Burkhead, 1994; R. Jenkins, Roanoke College, pers. comm.). Biologists working in this area in the future should be particularly observant for additional *Percina* hybrids. Further analyses of this and future suspected hybrids should seek to determine the direction of hybridization and whether crossings are one-time events or if introgression is ongoing. A targeted search for mitochondrial introgression between *P. rex* and *P. nevisense* in the Roanoke River also may be useful.

ACKNOWLEDGMENTS

I thank P. Angermeier, J. Philhower-Gillen, C. Williams, and B. Blood for sampling assistance during the discovery of the hybrid; D. Dutton and J. Printz for invaluable aid during laboratory work; P. Angermeier, J. Argentina, E. Hallerman, T. Near and J. Printz for helpful comments on the manuscript; and T. Roberts for logistical support during all phases of this project.

LITERATURE CITED

Anderson, E.C. 2003. NewHybrids Version 1.1. Available online at http://ib.berkeley.edu/labs/slatkin/eriq/software/software.htm#NewHybs.

Anderson, E.C., and E.A. Thompson. 2002. A model-based method for identifying species hybrids using multilocus genetic data. Genetics 160:1217-1229.

Benson, D.A., M.S. Boguski, D.J. Lipman, J. Ostell, B.F.F. Ouellette, B.A. Rapp, and D.L. Wheeler. 1999. GenBank. Nucleic Acids Research 27:38-43.

Bossu, C.M., and T.J. Near. 2009. Gene trees reveal repeated instances of mitochondrial DNA introgression in Orangethroat darters (Percidae: *Etheostoma*). Systematic Biology 58:114-129.

Dowling, T.E., and C.L. Secor. 1997. The role of hybridization and introgression in the diversification of animals. Annual Review of Ecology and Systematics 28:593-619.

Dutton, D.J., J.H. Roberts, P.L. Angermeier, and E.M. Hallerman. 2008. Microsatellite markers for endangered Roanoke logperch (*Percina rex*), and their potential utility for other species. Molecular Ecology Resources 8:831-834.

Echelle, A.A., and A.F. Echelle. 1997. Genetic introgression of endemic taxa by non-natives: a case study with Leon Springs pupfish and sheepshead minnow. Conservation Biology 11:153-161.

George, A.L., D.A. Neely, and R.L. Mayden. 2006. Conservation genetics of an imperiled riverine fish from eastern North America, the Blotchside logperch, *Percina burtoni* (Teleostei: Percidae). Copeia 2006:585-594.

Goodin, J.T., E.G. Maurakis, E.S. Perry, and W.S. Woolcott. 1998. Species recognition for *Percina nevisense* Cope (Actinopterygii: Percidae). Virginia Journal of Science 49:183-194.

Excoffier, L., G. Laval, and S. Schneider. 2005. Arlequin version 3.0: an integrated software package for population genetics data analysis. Evolutionary Bioinformatics 1:47-50.

Hubbs, C. 1955. Hybridization between fish species in nature. Systematic Zoology 4:1-20.

Jenkins, R.E., and N.M. Burkhead.1994. Freshwater fishes of Virginia. American Fisheries Society, Bethesda, MD. 1079 p.

Keck, B.P., and T.J. Near. 2009. Patterns of natural hybridization in darters (Percidae: Etheostomatinae). Copeia 2009:758-773.

Mayden, R.L. and L.M. Page. 1979. Systematics of *Percina roanoka* and *P. crassa*, with comparisons to *P. peltata* and *P. notogramma* (Pisces: Percidae). Copeia 1979:413-426.

Pritchard, J.K., M. Stephens, and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. Genetics 155:945-959.

Roberts, J.H., and A.E. Rosenberger. 2008. Threatened fishes of the world: *Percina rex* (Jordan and Evermann 1889) (Percidae). Environmental Biology of Fishes 83:439-440.

Ross, M.R., and T.M. Cavender. 1981. Morphological analyses of four experimental intergeneric cyprinid hybrid crosses. Copeia 1981:377-387.

Scribner, K.T., K.S. Page, and M.L. Bartron. 2001. Hybridization in freshwater fishes: a review of case studies and cytonuclear methods of biological inference. Reviews in Fish Biology and Fisheries 10:293-323.

Seehausen, O., J.M. Vanalphen, and F. Witte. 1997. Cichlid fish diversity threatened by eutrophication that curbs sexual selection. Science, 277:1808-1811.

Vähä, J-P., and C.R. Primmer. 2006. Efficiency of model-based Bayesian methods for detecting hybrid individuals under different hybridization scenarios and with different numbers of loci. Molecular Ecology 15:63-72.

TABLE 1. Results of Bayesian admixture analysis of the putative hybrid *Percina* specimen and 24 known-identity specimens at seven microsatellite markers.

Model 1		**Bayesian posterior probabilities**					
		Pure	Pure	F1	F2	F1 x *P. rex*	F1 x *P. nevisense*
Species	*n*	*P. rex*	*P. nevisense*	hybrid	hybrid	backcross	backcross
Hybrid	1	0.350	0.347	0.136	0.023	0.041	0.104
P. rex	15	>0.976	0.000	0.000	<0.001	<0.020	0.000
P. nevisense	4	0.000	>0.989	0.000	<0.001	0.000	<0.010
Model 2		**Bayesian posterior probabilities**					
		Pure	Pure	F1	F2	F1 x *P. rex*	F1 x *P. roanoka*
Species	*n*	*P. rex*	*P. roanoka*	hybrid	hybrid	backcross	backcross
Hybrid	1	0.962	0.009	0.002	0.016	0.014	0.004
P. rex	15	>0.996	0.000	0.000	<0.001	<0.004	0.000
P. roanoka	5	0.000	>0.990	0.000	<0.002	0.000	<0.008

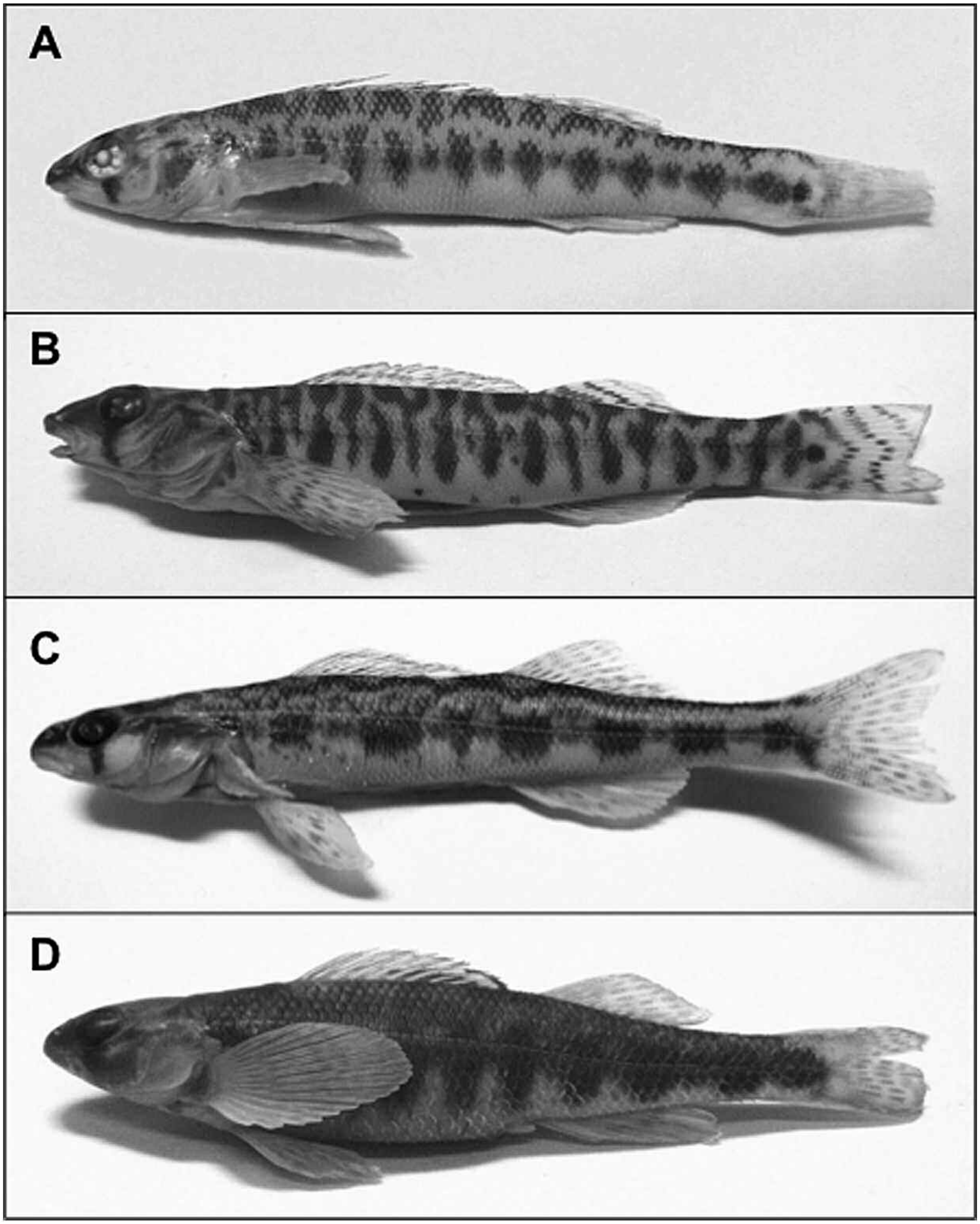


FIGURE 1. Photographs of all *Percina* species known to occur in the upper Roanoke River, including **A**) the putative hybrid specimen, **B**) *P. rex*, **C**) *P. nevisense*, **D**) *P. roanoka*. Pictured specimens were collected in the Roanoke River, Roanoke County, Virginia, and measured 58, 62, 76, and 58 mm total length respectively.

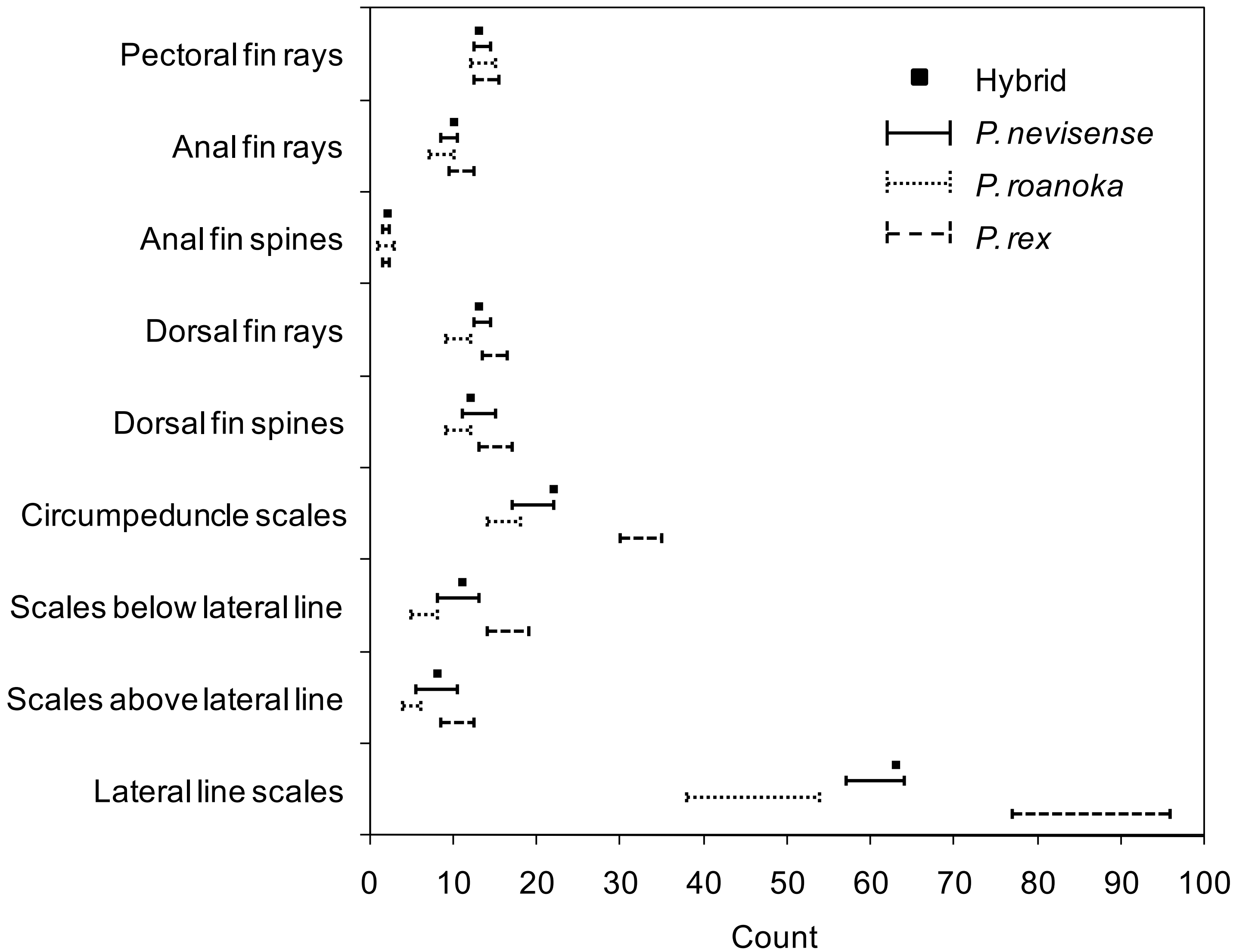


FIGURE 2. Measured values from the putative hybrid specimen and published ranges for *P. nevisense*, *P. roanoka*, and *P. rex* of counts for nine meristic characters.

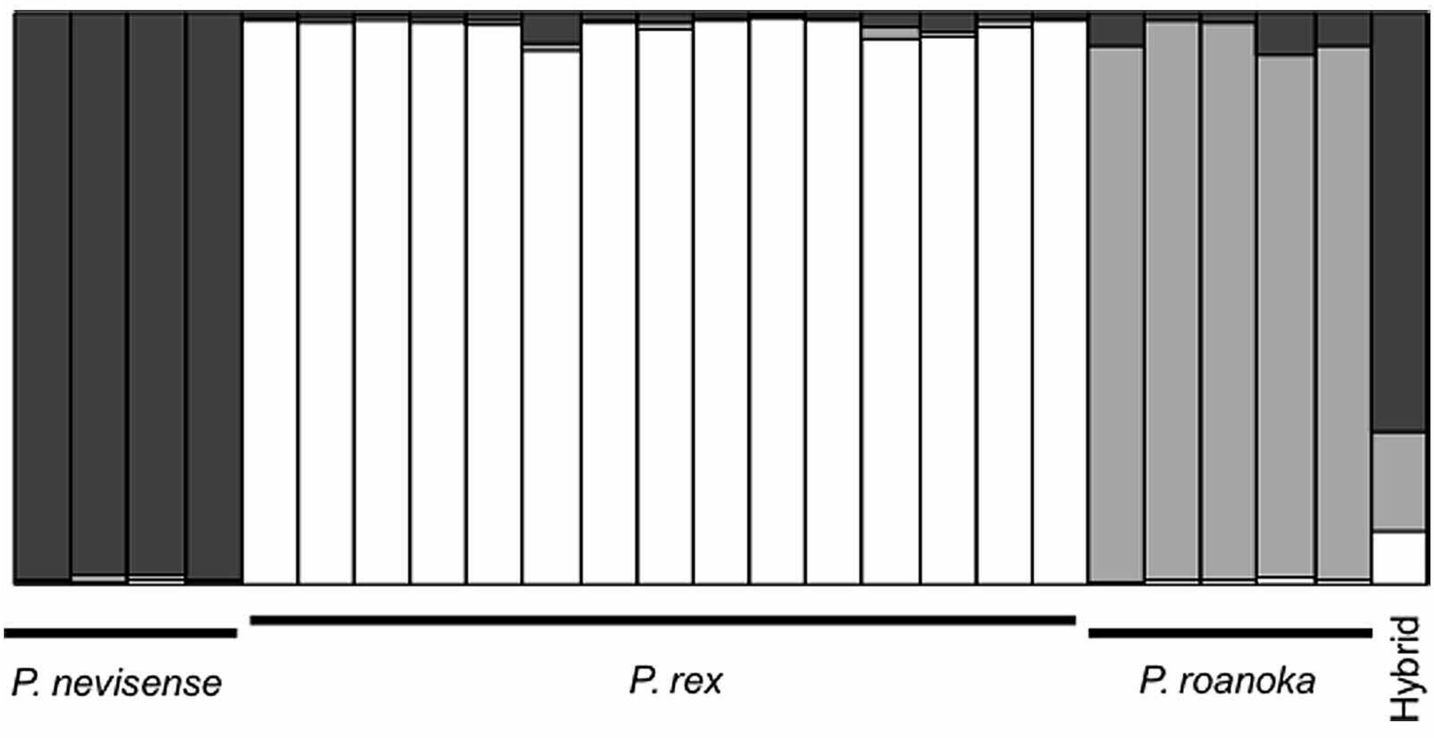


FIGURE 3. Results from STRUCTURE admixture analysis, showing Bayesian posterior probabilities of 25 individuals' origination from each of three inferred ancestral populations (represented by shading). Each individual is represented by a single horizontal bar.

Business Meeting
36th Annual Meeting of the Southeastern Fishes Council November 11th & 12th, 2010 Athens, Georgia

The 2010 business meeting of the Southeastern Fishes Council was called to order by Chair Gerry Dinkins at 4:21 p.m. One hundred and three people attended the meeting.

Report of the Secretary

Secretary Rebecca Blanton reported that the 2009 minutes were unanimously accepted by electronic vote of the membership with 25 members submitting a vote by e-mail. She noted that the 2009 minutes would be posted on the SFC website.

Report of the Treasurer

Treasurer Anna George presented the society's overall financial standing and the financial status of the 2010 meeting, noting that the society was financially steady. Details of the Treasurer's Report are given below. Jim Williams moved and Hank Bart seconded the motion to accept the report, which was unanimously approved by the membership.

Report of the Committees

Nominating Committee *– Carol Johnston.*
Chair Dinkins noted that Carol Johnston as chair-elect would be the head of the nominating committee, and beginning sometime next year she would be approaching individuals to assemble a slate of candidates for chair, secretary, and treasurer. He also explained that all three executive committee positions would be coming up for election because Treasurer George and Secretary Blanton were currently serving their second term. He thanked both George and Blanton for their service and asked that if anyone was interested in running for any of these offices, that they contact Carol Johnston.

Program Committee *– Mary Freeman, Anna George and Jim Williams.*
The committee provided brief comments on the 2010 meeting and Chair Dinkins thanked the committee and all others involved in organizing the 2010 meeting for their hard work.

Chair Dinkins also provided details on the location and dates for 2011 SFC Annual Meeting. He reminded the membership that through a previous agreement of the membership, every third year the meeting would be held Chattanooga, TN, thus the site for the 2011 meeting. He also noted that in the subsequent year the meeting would be held at a state park (such as Guntersville State Park), and then return again to a local college or university. Several members initiated discussion regarding the need to keep the meeting locale as centralized to the membership as possible. Hank Bart initiated discussion regarding hosting a future upcoming meeting in New Orleans, which would serve as a good venue because it would also be a place in which we could have a suitable memorial to Royal D. Suttkus.

Constitution Committee *– Bernie Kuhajda* (no action, no report)

Proceedings Committee *– David Neely and Chris Skelton*
Editor Neely discussed the status of online issues of the Proceedings and noted that he had received one and was anticipating a second manuscript for consideration for publication in the 2011 Proceedings. Brett Albanese requested that the password for accessing online issues of the Proceedings be re-distributed to the membership.

Awards Committee *– Anna George*
Anna George presented awards of appreciation to Mary Freeman and Jim Williams for organizing the 2011 meeting. Jake Schaefer was presented with an Amazon gift certificate in appreciation of his hard work on the SFC website.

Technical Advisory Committee –
Chair Dinkins noted that this was a newly created committee, and that the constitution stated that it must be composed of the following: the Executive Committee, two active field biologists from separate states, three active faculty members involved with southeastern fish research, and three people from the membership at large and must be assembled by the

Chair. The following people agreed to serve on the Technical Advisory Committee: Pat Rakes (Conservation Fisheries), Charlie Saylor (Tennessee Valley Authority), Steve Powers (Roanoke College), Ginny Adams (U. of Central Arkansas), Hank Bart (Tulane), Mel Warren (U.S. Forest Service), Jim Williams (U.S. Geological Survey, retired), and Brett Albanese (Georgia Department of Natural Resources).

***Membership Committee** – Rebecca Blanton*

Blanton reported a slight decline in membership in 2011. The total membership at the time of the meeting was 141 members including 5 life, 98 regular, 6 family, and 32 student memberships compared to a total of 167 members in the previous year and 192 in 2008. She noted however, that the total did not reflect those that had joined at or just prior to the meeting.

***Website Committee** – Jake Schaefer*

Schaefer provided a brief update on the SFC website, asking members to submit pictures and indicated that the online submission of abstracts worked well and that this mode of submission would be used for future meetings.

Old Business

Status of electronic only Proceedings.

Chair Dinkins noted that after careful consideration, the executive committee had decided that the Proceedings will not be a paperless publication (electronic only). Instead, the membership will be allowed to opt out of receiving the paper version, and receive only the electronic version is they so choose. A discussion was held on how this option would be instated. Treasurer George noted that an option would be added to the membership renewal/new membership forms to select preference of journal format.

Formal partnership with SARP.

Chair Dinkins explained to the membership that the partnership with SARP had been formalized.

Petition to list Elassoma alabamae.

Chair Dinkins noted a petition had been submitted to FWS, but no action had been taken as of the meeting date.

SFC history.

Jake Schaefer indicated that the history (1992 to present) of SFC officers, meeting places, etc. was nearly finished and would soon be posted on SFC webpage. Chair Dinkins confirmed that he was continuing to work on the historical information and would soon have this completed and ready for posting on the website.

Petition to list aquatic species by Center for Biological Diversity.

Jim Williams summarized this issue, providing an overview of SFC's review of list of fish species included. He explained that in conjunction with several members, SFC had proposed a 'trimmed' version of the list that removed those species of fishes that did not warrant immediate action for protection by the federal government. He noted that through their efforts, they had reduced the list by 20-30 species of fishes. This revised version was given to the CBD, but D. Neely noted that many, if not most, of the SFC recommendations had been ignored. Williams explained to the membership that the petition had not been filed as of the date of the meeting.

New Business

Monitoring the federal register.

Chair Dinkins noted that someone in the membership should monitor the federal register to identify actions that pertain to SFC. In doing so, the membership could be alerted to any actions that are related to the mission of the society and thus become involved as needed. The membership discussed the best mode of monitoring the register and disseminating information to the membership (email, Facebook, and other options were considered). Jim Williams asked U.S. Fish and Wildlife Service members present if SFC could be added to the e-mail list for Regions 4 and 5. Robin Goodloe of FWS said yes, but this would mean that members would also receive non-fish related actions as well. Williams volunteered to receive e-mails and pass on only those actions that were related to southeastern fishes to the membership.

Continuation of 'themed' meetings.

Chair Dinkins asked the membership to consider whether themes for subsequent meetings should be continued or if we should eliminate themes and have only submitted papers in the oral presentation sessions. This discussion was raised due to the large number of papers submitted for the 2010 orals sessions, which resulted in several members being asked to present as posters. He noted that the executive committee felt strongly that a symposium theme be continued as part of the annual meeting, rather than submitted papers only, and that the theme session take up the first half of the first morning session (1/8 of total meeting time). This decision was supported by the membership.

Payment of meeting registration.

Mel Warren asked if the cost of membership could be included in the meeting registration cost. Treasure George explained that doing such caused confusion for members as to which year was being paid. She indicated that the SFC membership was an annual membership and should be renewed in January each year. She concluded that it would be possible to add an option to pay for the meeting registration and membership simultaneously, but members would have to indicate which year they were paying for and that she would revise the payment form to include a way of indicating the year of membership paid.

Passing of Dr. Royal D. Suttkus.

Chair Dinkins noted the passing of one of the society's founding members, Dr. Royal D. Suttkus in December, and Hank Bart's obituary that appeared in the spring issue of Copeia.

Bart told the membership that he was working with the family of Dr. Suttkus to plan a memorial service and celebration of his life. As the organizer of the service, Bart asked the membership to consider making a financial contribution to help pay for the expenses. Carol Johnston suggested that a future SFC meeting and memorial to Suttkus be held in conjunction in New Orleans, LA. Anna George expressed concerns with holding a meeting in New Orleans, because it would move the meeting from a central location and possibly prohibit many members from attending. Greg Moyer suggested that the society have a 'Suttkus-themed' meeting, but to do so in one of the selected central locations.

Establishing a relationship with Patagonia –

Anna George told the membership that she had been approached by Patagonia to see if the society might have an interest in establishing a relationship with them. Patagonia had seen the Desperate Dozen list produced by the society and was interested in an updated and possibly a geographically expanded list. Discussion ensued regarding the potential financial and/or public outreach benefits to the society. George noted that it would not likely involve a financial gain, but would be more of a public relations outreach relationship. Mollie Cashner noted that this relationship may provide a long-term potential for money through recognition of SFC on the Patagonia website. Discussion was also initiated regarding the request to modify or expand the original Desperate Dozen list. The conclusion by the membership was to use the existing Desperate Dozen publication and restrict our geographic focus to the southeastern US. George suggested that we indicate that SFC would regularly review and update or add to the list as needed. She also asked that the Technical Advisory Committee review the potential collaboration.

New FWS position and upcoming federal register actions involving SFC.

Cindy Williams announced the creation of a new FWS position that will be housed at the Tennessee Aquarium in Chattanooga. She also requested that the SFC Technical Advisory Committee look over the federal register and provide comments on the recent listing of the Atlantic Sturgeon. She also indicated that FWS may propose expansion of some refuges and may request SFC to provide comments.

Fishes in need reports.

Anna George presented a new initiative in collaboration with SARP to identify and provide updated species accounts for Tier I, II, and III species in the Mobile and Cumberland drainages of the southeastern United States. She identified experts and asked those identified to volunteer to write a species account, including conservation status, threats, and needed conservation actions of each identified species. Brett Albanese asked the purpose of this endeavor. George replied that it would provide updated information of threatened and endangered fishes of these regions to the USFWS and she also discussed the possible public outreach potential of the project.

State reports

Chair Dinkins and Editor Neely noted the State Reports would soon be posted on the SFC webpage and would only be published online in the future.

The meeting was adjourned by Chair Dinkins at 5:31 p.m.

Respectfully submitted, Secretary Rebecca Blanton Johansen

2010 Treasurer's Report for the Southeastern Fishes Council Prepared by Anna George

Starting Balance	**$23,208.90**
1 January 2010	
EXPENSES	
2010 Annual Meeting	
Room Rental	$1475.00
Catering	$5247.47
Student Awards	$600.00
Invited Speaker Costs	$496.52
Entertainment	$300.00
Gifts and Other Fees	$233.67
Proceedings Printing	$1397.00
Proceedings Mailing	$449.88
Paypal Fees	$371.87
Miscellaneous Fees	$20.00
TOTAL EXPENSES	**$10,591.41**
INCOME	
Memberships	
112 regular at $30	$3360.00
47 student at $15	$705.00
7 family at $40	$280.00
2 lifetime at $400	$800.00
2010 Meeting Registration	$11,220.00
Additional Contributions	$750.00
Proceedings Sales	$1090.00
TOTAL INCOME	**$18,205.00**
ENDING BALANCE	**$30,822.49**

Southeastern Fishes Council Proceedings

INFORMATION FOR CONTRIBUTORS

The primary purpose of the *Proceedings* is to publish peer-reviewed research papers and critical reviews of activities; regional reports and notes; and other pertinent information pertaining to the biology and conservation of southeastern fishes. The *Proceedings* is also an outlet for range extensions, distributions, and status papers, covering ecology and conservation ichthyology. Life history studies, faunal surveys, management issues, behavior, genetics and taxonomy of southeastern fishes are appropriate topics for papers in the *Proceedings*. Review papers or information on imperiled waters or fishes are particularly appropriate.

Manuscripts can be submitted electronically via email (send to: dave.neely@gmail.com) or mailed as hard copies to the address below. Mailed hard copies should be submitted in triplicate. A good guide for manuscript preparation is the Sixth Edition of the *CBE Style Manual* available form the Council of Biology Editors, One Illinois Center, Suite 200, 111 East Wacker Drive, Chicago, IL 60601-4298.

The entire manuscript including the Abstract (required for longer articles), Introduction, Methods, Results, Discussion, Acknowledgments, Literature Cited, Appendices, Tables, and Figure Legends must be double-spaced. The title, author's name and author's address (including fax number and email address for corresponding author) should be centered on the first page. Indicate a suggested running head of less than ten words at the bottom of the first page. An Abstract (if necessary) will be placed at the beginning of the text. Acknowledgments will be cited in the text immediately before the Literature Cited. All references cited in the paper will follow the standard format of using the last name of the author(s) followed by the year of publication of the paper. In the Literature Cited, the references will be alphabetical by the author's last name and chronological under a single authorship. Literature cited should be standardized and abbreviated, using the *World List of Aquatic Sciences And Fisheries Serial Titles* or guidelines in *CBE Manual for Authors, Editors, and Publishers, 6th edition* for journals not included in the World List.

Tables should be typed on a separate page, consecutively numbered and should have a short descriptive heading. Figures (to include maps, graphs, charts, drawings and photographs) should be consecutively numbered and if grouped as one figure each part block lettered in the lower left corner. Computer-generated graphics should be high quality prints; for drawings, high quality prints or photocopies are preferred to the original line art. Legends for figures must be on a separate sheet. When possible, tables and figures will be reduced to one column width (3.5 in), so lettering on figures should be of appropriate size. Color figures can be printed at the author's expense.

Manuscripts will be subject to editing and will be reviewed by at least two anonymous persons knowledgeable in the subject matter. The edited manuscript and page proofs will be furnished to the author. Upon returning the reviewed and corrected manuscript to the editor, a PC disk copy of the final form of the text, tables and computer-generated graphics is also requested. Specific formatting information for the disk will be sent to the author with the edited manuscript. Reprints can be ordered at the time of printing, and will be supplied to the author at the cost of printing.

Regional reports, news notes and other short communications will also be edited and included when possible in the next number.

Only manuscripts from members of The Southeastern Fishes Council will be considered for publication. There is no charge for publishing in the *Proceedings*. All manuscripts and short communications should be sent to the editor:

Dave Neely, Editor
Southeastern Fishes Council Proceedings
Tennessee Aquarium Conservation Institute
1 Broad Street
Chattanooga, TN 37402
Phone: (415) 608-9227
Email: dave.neely@gmail.com
Southeastern Fishes Council Web Site:
http://www.sefishescouncil.org

Proceedings is a publication of the Southeastern Fishes Council, Inc., and is published in Milledgeville, Georgia. Officers are Carol Johnston, Chair; Ginny Adams, Chair-Elect; Gerry Dinkins, Immediate Past Chair; Mollie Cashner, Secretary; Steve Rider, Treasurer; and Jake Schaefer, Webmaster. Managing Editor for the *Proceedings* is David Neely, Southeastern Fishes Council Proceedings, Tennessee Aquarium Conservation Institute, 1 Broad Street, Chattanooga, TN 37402, Phone: (415) 608-9227, Email: dave.neely@gmail.com; Associate Editor is Christopher E. Skelton, Department of Biological and Environmental Sciences, Georgia College & State University, Milledgeville, GA 31061, (478) 445-2440, chris.skelton@gcsu.edu. *Proceedings* is printed on recycled paper.